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# THE NEW PHYTOLOGIST

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## THE CARBON/NITROGEN RATIO IN THE WHEAT PLANT

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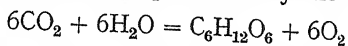
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### HISTORICAL

THE assimilatory reaction represented by the equation



has been called the primary reaction of life. The carbohydrate so formed is either used by the plant as building material for cell walls and the excess stored as such or it is used in the nitrogen metabolism. Whether carbohydrates themselves are built up into the simpler proteins or whether they act merely as catalysts in the reaction is still a debatable question; but the apparent dependence of protein synthesis on carbohydrate synthesis, whether direct or indirect, suggests some kind of correlation between them.

It is many years since growth was recognised as a purely chemical process, while recent biologists such as Loeb (38), Blackman (2), and Robertson (51) have amplified this with the concept that the process is explicable as an autocatalytic reaction, which may be controlled by varying either the internal or external conditions.

From this viewpoint such criteria as the carbon and nitrogen relations become standards whereby we may measure life processes and assess vitality.

The realisation of the possible significance of the carbon/nitrogen balance in plant growth seems to have begun early<sup>1</sup>. Zacharias (37) records that Lonicerus concluded that an excess of nourishment leads to a very marked extension of vegetative branches but that no fruit will be borne in these circumstances.

Of the early writers Klebs (31) perhaps did most in correlating the type of response of a plant with the determining environmental factor. Working on *Sempervivum*, he came to the conclusion that light intensity coupled with the supply of available nitrates is the controlling factor in determining the transition of a plant from the purely vegetative to the reproductive condition. In his later work, he divided the process of flower formation into three parts, the production of conditions of ripeness to flower, the development of flower primordia and the production of flowers proper, with the elongation of the axis. In the first and third steps light is the controlling factor, not as a direct agent so much as through its photosynthetic action. Here we have the first recognition of the balance of carbon assimilation and mineral absorption.

In the present century Murneek (39), working on the tomato, showed that the autocatalytic curve can be turned into a straight line if the sexual phase of the plant is suppressed. In the absence of any proof of an internal secretory system in plants, he explains cyclic growth as an outcome of different stages of localised nutrition. He suggests further that of all the nutritive factors, it is the proportion of carbon to nitrogen which is the prime factor underlying growth.

Petri (43, 44) in his investigations on the olive, came to the idea that although a deficiency in nitrogen relative to the carbohydrate supply constitutes a stimulus to prolific flower formation, a continued decrease in nitrogen leads to complete abortion of the young ovaries.

In 1918 the most comprehensive work on the correlation between internal conditions and phenomena of growth appeared. This was the work on the carbon/nitrogen relation in the tomato by Kraus and Kraybill (32) in America. From a large number of experiments they attempted to deduce the relation between the amount of vegetative growth, flower production and fruit setting and the amount

<sup>1</sup> *Vide* the parable of the unfruitful fig tree for an account of the early recognition of root pruning as an aid to fruiting.

of the available nitrogen and carbon reserve within the plant. They recognise four main conditions:

If a plant is provided with abundant moisture and nitrates coupled with a low carbohydrate supply it is weakly vegetative and non-fruitful (Class I).

On the other extreme, if it has very little nitrogen and an abundance of carbohydrate the same poorness of vegetation and suppression of fruitfulness is found (Class IV).

Between these two limits there are two other classes. With abundant available nitrates and a medium carbohydrate supply, vegetation becomes prolific but the plant remains sterile (Class II). Starting from this condition, a decrease in nitrogen with a slight increase in carbohydrate reserve, causes less vigorous growth but fruitfulness is induced (Class III).

Hence their law "Fruitfulness is associated neither with the highest nitrates nor the highest carbohydrates but with a condition of balance between them."

They also recognise the fact that the conditions for the initiation of flower primordia are probably not the same as for fruit setting.

The horticultural significance of these results was immediately apparent. Methods such as pruning, ringing, and manuring, once carried out mechanically by traditional ideas, now assumed the proportions of distinct physiological factors, inasmuch as they controlled either the amount of nitrate entering the plant or the amount of its photosynthetic activity. Particular attention was paid to the application of this law to the apple tree, a perennial with a marked tendency to a two-year cycle, comprising the formation of the fruiting spurs one year and the development of the flower and fruit the next. Work of a general nature had been carried out by Butler, Smith and Curry<sup>(3)</sup> in 1917, who worked out with great care the distribution of nutrients throughout the tree at different periods during this two-year cycle, but no stress was laid by them upon the value of either the carbon or the nitrogen content in any growth condition.

When dealing with the apple tree, the perennial nature of the plant made the problem more complicated than in the tomato, where different parts of the stem reach different stages at the same time, whereas in the apple every spur bearing fruit is in the same stage at the same time. This led to the conception of the "individuality of the spur" and most workers have utilised this conception in their researches since it necessarily reduced the number of trees for the

experiment and so cut down variability errors. However, they realised too, that to a certain extent this creed was fallacious since at any time a spur may draw on the entire tree or any part of it to supplement its own reserves.

Hooker and Bradford<sup>(20)</sup> experimented with fertile and vegetative shoots, measuring their growth and estimating the chemical differences between the fruiting and the non-fruiting spurs, the bark of the branch, the limb of the tree and the trunk, and came to the conclusion that although in many cases the spur condition alone may be decisive, the conditions back in the tree are very often important and decisive of the spur's performance.

H. D. Hooker<sup>(22, 23)</sup> himself found the seasonal changes in the potassium, sugar, starch and nitrogen content in three types of apple spurs—viz.: (1) spurs that blossomed and bore fruit; (2) spurs that did not blossom, but which developed fruit buds; (3) spurs that neither blossomed nor developed fruit buds—to be distinct and characteristic. Correlating the carbon and the nitrogen with the stage of development, he found that beginning with a low nitrogen and a high carbon content at the outset of the non-blooming year, there is a constant winter loss of both elements up to the formation of the terminal buds. The spring and summer leafy stages of the tree by increasing assimilation, tend to build up carbon reserves, while the nitrogen content remains low. These conditions ensure fruit bud formation. When once the buds are formed, the C/N conditions inside the spur change rapidly, the nitrogen accumulating up to its highest concentration. As the low C/N ratio resulted in much vegetative growth the previous spring, the increased ratio now induces flowering and fruit production, with the consequent depletion of the store of both carbon and nitrogen, until at the end of the season the low C/N conditions characteristic of vegetative activity are reached. This work confirms that of Kraus and Kraybill<sup>(32)</sup> and explains the biennial fruiting habit of most apple trees upon a purely nutritive basis.

Hooker<sup>(22, 23)</sup> applied his laboratory results to orchards by testing the effects of nitrogenous manuring at different periods of the year. He considers the age of a tree to be an important factor in determining the effects of fertilisers, since the initial C/N ratio must be considered. The time of the year at which the application is made is also highly important. Spring applications in the off year, by increasing the C/N ratio, do not favour fruit bud differentiation, although applications at the same time the following season increase



the nitrogen accumulation in the spur and consequently materially increase the set of fruit.

Fischer (12), too, formulated a similar theory, recognising the fact that when the nitrogen content was high in relation to carbon, vegetative activities were the result, whereas a high C/N ratio due either to accumulated carbon or reduced nitrogen favoured reproduction.

Magness (40) and Roberts (48), with apple tree material, came to the conclusion that the number of fruit buds formed is directly proportional to the number of adjacent leaves, and that factors causing the removal of leaves near the spur cause a decrease in the number of fruit buds. This also has an important bearing on the practice of defoliation and is directly in line with this work, since the loss of leaves would necessarily decrease the assimilatory activities and consequently carbon accumulation.

Wiggans (60) after a five years' study on apple trees considers the spur absolutely as an individual and, while stressing the value of nitrogen applications in determining the size of the tree, the development of its fruiting wood, and the production of blossoms, points out that the sap of fruiting spurs is higher in carbon than that of non-fruiting spurs.

Remy-Bonn (50), however, is disinclined to support the carbohydrate accumulation theory of spur formation and holds that nitrogen concentration is the prime factor.

Harvey and Murneek (24), examining the effects of defoliation on the composition of apple spurs, were able to explain this apparent primary significance of the nitrogen concentration. They show that spurs, defoliated in mid June, have a lessened C/N ratio due to increased nitrogen and decreased carbon. Thus, in general, decreased flowering results from defoliation. However, if the carbon is already very high as it would be in an old plant, a lowering of the carbon by defoliation would change it from Class IV to Class III—the reproductive class—of Kraus and Kraybill. This, of course, makes nitrogen appear to be the prevailing factor.

They also comment upon the fact that defoliation, while reducing the fruit bud formation by nearly 50 per cent., reduces the C/N ratio only by approximately 20 per cent., and conclude with the assumption that the carbon/nitrogen balance was much less significant in apple blossom formation than it had been in determining reproduction in herbaceous plants. However, they were bound to recognise it as among the controlling factors.

The second part of their work consisted of the correlation of fruit productiveness with defoliation in spring. Their chemical analyses showed that April defoliation increased the C/N by a decrease in the nitrogen, and an increase in the carbon, while practical results showed a proportionally equivalent decrease in the set of fruit. Like Hooker<sup>(22, 23)</sup> they state that fruit setting is greatest when the C/N is low.

Later, Murneek<sup>(30)</sup> was able to show that bearing spurs attained their nitrogen maximum at fruit setting whilst exhibiting a minimum of carbohydrates, while Harvey<sup>(25)</sup> extended the investigation to cover the effects of ringing and defoliation separately and in combination and at different seasons. By decreasing the amounts of carbon and nitrogen in the spur, defoliation changes the condition of the shoot back to that of a younger shoot, but the actual result of the process depends on the time of the year at which it is performed. If defoliation is carried out during the active growing period, a distinct check to growth is experienced, since the carbon is then limited, but when the carbon is high and the nitrogen limited as would occur in the early part of the season, defoliation by lowering the C/N ratio maintains vegetative activity in the plant and may even accelerate the growth rate.

Roberts<sup>(49)</sup>, in 1921 gave excellent support to Kraus and Kraybill's work by showing that a very high C/N ratio, if caused by a very low nitrogen content, is deleterious to growth and reproduction. On the other hand, a relation in which the nitrogen is very high leads to vigorous vegetation and no fruit; while it is the intermediate values both of carbon and nitrogen which give good vegetative response and fruitfulness. He also went further to point out that trees store nitrates one year for use the next when the available nitrogen is low. He applies this to the transplanting of trees from poor soil to richly nitrogenous soil and *vice versa*.

Other work showed that disbudding apple trees reduces growth very little, but reduces the number of spurs formed by 50 per cent. However, the number of fruit buds per spur is unchanged, while the fruit set is materially increased. The explanation of these results is clear and in accordance with the previous work. Disbudding, by reducing the foliage, would simultaneously reduce the carbon-accumulation and increase proportionally the amount of nitrogen. These conditions are such that fruit spur formation would be checked but these same conditions are those which favour the fruit set.

Gourley<sup>(13)</sup> gives us an account of the effects of many different

fertilisers and soil and air temperature upon growth of apple trees in general, but his most valuable observation was that in alternately bearing trees there is a heavier storage of starch in the medullary rays and pith when the tree has formed fruit buds. On the average he obtained about 4 per cent. greater specific gravity in the twigs and buds in winter conditions where fruit buds were formed.

Gardner<sup>(15)</sup> applied the work already published on apple trees to the strawberry and found that the best time to apply fertilisers to this plant would be late summer and early autumn since the poor conditions of the soil at that time, tend to the greatest accumulation of starch and sugar at the time of fruit-bud differentiation, so producing the maximum flower clusters and berries; but when once formed, increase in nitrogen increases fruit set and the quality of the fruit.

An interesting extension of Gardner's<sup>(15)</sup> work is the application of the C/N ratio to sex differentiation. Results of strawberry cultivation go to show that a low carbon content at the time of fruit-bud initiation leads to the suppression of the male organs in normally hermaphroditic flowers, which suggests to him that low carbon is associated with female differentiation, high carbon with male, intermediate values giving hermaphrodite forms.

Smith<sup>(52)</sup>, working on Maize, found by analysis that while in the earlier stages of development the leaves were rich in nitrogen, after ear formation the nitrogen in the leaves and stem decreased and accumulated in the ear until at maturity the ears contained about one half the amount of the whole plant.

This is in agreement with the results of Hornberger<sup>(26)</sup>, who showed that following a period of rapid absorption of most of the nutrient elements, at the time of ear formation, there is a decrease up to early maturity, whereon follows a marked increase during the ripening of the ear and a loss in everything until complete maturity.

There has been a considerable amount of purely physiological research following the lines of the C/N ratio theory as formulated by Kraus and Kraybill<sup>(32)</sup>. Very soon after their papers, Woo<sup>(59)</sup> published a report on the chemical constituents of *Amaranthus retroflexus*, a pernicious weed, reputed to store nitrogen and so obtain a material advantage over its competitors. Although in general supporting Kraus and Kraybill's results he also points out that the theory has certain limitations since the C/N is not a mathematical or physical constant but that it varies considerably, sometimes decreasing as the plant progresses, and where the sub-stratum is

liable to alteration in concentration, the ratio might vary, too, throughout the day and at different times of the seasons. However, regardless of the constancy of the ratio, his curves for root, stem and leaf show the reciprocal relations of carbon and nitrogen, and he himself stated that his work is no proof that the ratio does not exist. He was also able to show that *Amaranthus* flowered even when the nitrogen content was high and the C/N low, and was forced to conclude that a different range of the C/N ratio is required to produce the same range of effects in different plants.

Campbell(6), however, working on the same plant found no trace of nitrate in any specimen at full maturity and his nitrate curve thus rose gradually from early seedling stage to bloom stage where it began to fall, reaching zero at full maturity.

A year later, Woo's(59) conclusion was justified by Gurjar(30) on Tomato. Believing that it is the supply of nitrogen which determines the relative proportion of carbohydrates and proteins in this plant, he shows that although the C/N ratio may be as high as 19 and as low as 2, fruiting will occur only at values of 4-6. These values are characteristic of this plant and are vastly different from the high values in apple spurs as cited by Harvey, Murneek and others. Besides this aspect of the problem, the same author introduces the difficult and complex question of the correlation between metabolism and this C/N ratio. Fischer(12) had suggested that since the carbon was fixed from the CO<sub>2</sub> of the air by photosynthesis anything affecting this process would affect the ratio. Gurjar(30) shows that photosynthesis varies inversely as the value of the C/N ratio, while the sister process of respiration varies with it directly. Also the actual amount of respiration varies with the temperature and the initial carbon content of the plant.

This laid the foundation stone for much work on the interrelation between metabolism and growth response due to the C/N relation.

One of the factors controlling photosynthesis is light, and Nightingale(42), working extensively on Tomato, Salvia, Buckwheat, Soya Beans and Radishes, found that their carbon content depends upon the intensity of lighting by the control of their photosynthetic activity. Moreover, Nightingale(42) propounded the theory that carbohydrates form a base in the synthesis of the simpler proteins from nitrates, and if the carbohydrates are limited the nitrates accumulate. However, these accumulated nitrates did not affect the type of development, particularly flowering, as Woo had similarly found in *Amaranthus*. Thus Nightingale supposed that the significant

ratio determining growth was that of carbohydrate to insoluble nitrogen. This ratio was worked out, and plants subjected to different lengths of exposure to light. The carbon content was thus altered and the growth responses then accorded with the requirements of Kraus and Kraybill's theory. Later, in conjunction with Gourley (14), this work was extended over many horticultural plants. Their general conclusions were that although species and varieties differed in their responses, the response was always in the one direction that shade suppressed fruit-bud formation and blossoming, and in extreme cases inhibited it altogether, while in every case where flowering occurred the process was delayed from a few days to more than one month.

Kraybill (33) shaded apple trees and found in these trees a direct variation in the C/N ratio in response to degrees of shading. Thus, by increasing the intake of moisture and nitrogen, and reducing the opportunity for carbon synthesis, a condition is set up where both the carbon and nitrogen are used for vegetative growth, and no reserve stored up and thus trees failed to form fruit spurs. Ringing had an opposite effect to shading chiefly by preventing the translocation of carbohydrates to the roots and so causing carbon accumulation.

Not only was the effect of light intensity determined, but many workers turned their attention to the duration of daily exposure to light.

Garner and Allard (17), as a result of experiments with many plants, state that "Of the various factors of the environment which affect plant life, the length of day is unique in its action on sexual reproduction. Except under such extreme ranges as would be totally destructive or at least highly injurious to the general well being of the plant, the results of differences of temperature, water supply, and light intensity as far as concerns sexual reproduction appear to be at most merely an acceleration or retardation, as the case may be, while the seasonal length of day may induce definite expression, initiating or inhibiting the reproductive processes dependent upon the species." They found that some plants would respond to a "short day" and others to a "long day"; but if the favourable length of day were not present no sexual phase would be initiated. Explaining this on the basis of the C/N ratio theory, it is obvious that the amount of carbon assimilation should be directly proportional to the length of light exposure regardless of intensity. Knight (34) is inclined to doubt this statement, on the grounds that

there is no evidence to show whether the differences in total radiation were or were not accompanied by differences in photosynthetic activity, light in those experiments possibly never being a limiting factor in the carbon production. He states that a correlation of this work with chemical analysis is highly desirable.

Gilbert(18) worked to secure information as to the metabolic conditions within the plant as it approached flowering. Using *Xanthium* he varied the temperature and the length of day and in every case was able to show that, although under these varied conditions, the actual magnitude of the C/N ratio varied so that to no single ratio could be assigned the causative role in the formation of flower primordia, the trend of the ratio in all cases was the same, showing that when reproduction does begin the same metabolic conditions occur inside the plant as far as the C/N ratio indicates. His graph for high temperature, short day plants, sampled between the seventh day and the sixteenth day, when buds appeared on the twelfth day runs as shown in Fig. 1.

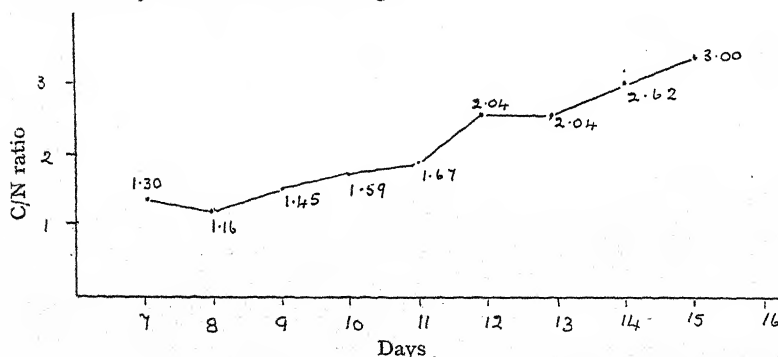


Fig. 1. Gilbert's graph showing march of C/N ratio in *Xanthium*.

Prior to this Summers(53) surveyed all the governing factors in bud formation, paying particular attention to the memoir of Butler, Smith, and Curry(3) on the composition of the apple tree. He concludes in favour of a direct causal connection between bud formation and the C/N relation.

Duley and Millar(11) show that the total weight of nitrogen increases throughout the growth period but to so small an extent that there is a steady drop in the percentage of this element. No stress was laid on the carbon content or the C/N ratio by these authors.

Similar figures are quoted by Jones and Husten(29) for Maize.

## *The Carbon/Nitrogen Ratio in the Wheat Plant*    II

Shull records the interesting results of Prianschnikow<sup>(45)</sup> who considers that nitrogen metabolism is a reversible chemical reaction running from proteins and amino-acids by oxidation and secondary synthesis to amides and from these by further oxidation to ammonia. This is the process when the carbohydrate value is low. If, however, carbohydrate is introduced into the system the process immediately reverses, ammonia being synthesised to amides and hence to amino-acids and proteins. If nitrates are the source of the plant nitrogen, the process is the same, the nitrates being first broken down to ammonia, and the amount of carbohydrate present in the plant determines the flow of metabolism and nitrogen storage. Hence the balance of C/N assumes a fundamental importance and justifies the explanation of growth responses on the basis of this ratio.

A similar thought seems to have been in the mind of Hartwell<sup>(28)</sup>, when he found that starch congestion, or factors associated with it, retarded growth in the aerial parts of plants, and stated that although these factors did not seem to interfere with photosynthesis, they may have influenced metabolism in some way.

Up to the present the investigations have chiefly been attempts to correlate horticultural practice with pure physiology, and such practices as pruning, ringing, defoliation and nitrogenous manuring have been explained on the basis of their effects upon the carbon and nitrogen metabolism of the plant. Similarly the effects of light, shading, short and long day upon flower production and size and vigour of the plant have displayed a relationship to the carbon and nitrogen content of the plant while by some writers this C/N relation has been given great prominence as a prime factor in determining vegetative vigour, reproductive activities or even sex differentiation.

It was felt that perhaps the best way to trace the relationship of these two elements to growth and reproduction, would be to trace the C/N ratio which occurs throughout the life history, from embryo to embryo, of a normal annual plant, growing under normal conditions and let the plant show for itself what internal conditions stimulate growth responses. With this end in view, the present work was begun in 1925, at Cardiff University College under the direction of Professor McLean, to whom my thanks are offered for many helpful suggestions.

## EXPERIMENTAL PROCEDURE

For the experiment wheat was chosen, offering as it does the advantages of easy cultivation, fairly rapid growth, the availability of pure lines and the possibility of the comparison of strains with different normal growth periods.

To Professor Percival of Reading, I am indebted for the supply of pure line seeds from a single ear of three varieties of wheat of varying growth periods, "Starling," a winter wheat with a life period of normally approximately eight to nine months or more, "Marquis," an American spring wheat completing its life history in five months, and "Nevin Bearded," an English spring wheat, somewhat intermediate between the two.

It was hoped that by comparison of the carbon and nitrogen contents of these three strains under similar cultural conditions, at similar time intervals and at equivalent developmental stages respectively, definite conclusions might be formed as to the relation of length of growth period to chemical constitution, and the possible controlling effect of the latter on the former.

Since the seeds were from pure lines of wheat and selected from one ear only, individual inherited variations were reduced to a minimum. Trelease (57), however, has pointed out that the size of both aerial and underground parts of "Marquis" wheat plants is directly proportional to the size of the seed, particularly during the seedling stages. With this in mind each seed was weighed before sowing and a record kept of each seed weight for future reference. The seeds with the exception of four of similar weight were placed in a Petri dish on moistened blotting paper and watered with distilled water (nitrate free) only. The four remaining seeds were dried to a constant weight in a steam oven at 100° C. and the embryos dissected out. Two were used for the carbon and two for the nitrogen estimation. The winter wheat was sown on December 25th, and four days later the embryos were prominently swollen. Four seeds, again of similar weight and similar development, were removed, dried as before and the embryos investigated for carbon and nitrogen content. Similar sets of material were taken on the seventh and tenth days after sowing. On the tenth day the seedlings were planted in glazed earthenware pots of diameter 12 in. and height 14 in., filled with quartz sand, which had been washed with running water until free from salts. The following nutritive culture was



given every third day, soft tap water supplying their needs between culture waterings:

- 1.0 grm. potassium nitrate
- 5 grm. ferrous phosphate
- 25 grm. magnesium sulphate
- 25 grm. calcium sulphate
- 1.5 litres distilled water.

This was the formula first used but after a few applications it was evident that the food supply was inadequate, so the dilution was decreased, viz. one litre of water substituted for 1.5 litres. This was found to be satisfactory as far as the obvious general health and development was concerned, though lacking in nitrogen as will be shown later.

These plants were grown under glass at Roath Park, some distance from the laboratory<sup>1</sup>. This led to the problem of transporting the materials to the laboratory with as little loss as possible in carbon content due to respiration probably increased by conditions of transit. It was decided to kill the plants on gathering with a suitable toxic agent. The most complete method of freezing was impracticable under the conditions, so the effect of anaesthetics had to be tried. Thoday(56) has shown that chloroform first of all increases the respiration enormously and only after twenty minutes depresses it to zero. Ether has been shown to have a similar effect. By comparative experiments with killed and unkilld material strong xylol vapour was found to be effective in quickly depressing the respiration to a minimum. Thus for purposes of collecting, jars were prepared in which cotton wool pads soaked in xylol were placed some little time previous to use and kept tightly corked. When required for use the pads were removed.

Another factor demanding consideration was the time of day at which the samples were taken. Since the carbon compounds are direct products of photosynthesis, a metabolic process depending upon external factors, there is necessarily a fluctuation throughout the day in the actual amount of carbon present in any one part of the plant. Davis(9) has shown that in the potato leaf, saccharose increases in amount from sunrise to 2.0 p.m., following approximately the curve of temperature. It then falls during the rest of the day. The effect of light intensity on photosynthesis is similar to that of temperature, so under greenhouse conditions with approximately constant temperature it is fairly safe to assume that the carbon curve will show the daily linear rise and fall shown by Davis(9).

<sup>1</sup> All the plants remained healthy and free from fungal attack throughout.

Moreover, Chibnall(7) has shown that there is a distinct diurnal variation in the protein nitrogen content of runner bean leaves, comprising a fall in protein nitrogen at night of 1.8 per cent. with a decrease in non-protein nitrogen of 9.0 per cent. The ammonia and amide nitrogen of asparagine remained unchanged in these leaves, showing that the products of protein decomposition must have been undoubtedly translocated to other parts of the plant. Mothes(41), Stiles(55), and other physiologists confirm this.

Thus in order to keep the results comparable, all material was collected at 3 p.m., a time at which protein disintegration is least active and carbon assimilation just past its maximum.

The killed material was freed of sand, divided into root, stem, leaf, etc. and dried to constant weight at 100° C. It was then stored in Petri dishes until estimation could be made.

Many factors had to be considered in the choice of apparatus for the carbon and nitrogen estimations. Hitherto most investigators have worked in great detail upon the carbohydrate content of the plants under consideration, carefully distinguishing between sugars, hydrolysable and unhydrolysable; polysaccharides, starches, etc. while similarly the nitrogenous compounds have been estimated as proteins, amides, amino acids, nitrates, nitrites, and ammonia compounds. However, owing to the number of estimations to be made in this work, it was thought that such detail would unnecessarily complicate and eventually obscure the main point, so it was decided to confine the work to total carbon only.

Thus in the significance of the term C/N ratio this work differs from any of the previous investigations. The term C/N ratio has been used loosely and its significance depends upon the particular author. Thus to many it is the carbohydrate/nitrogen ratio, to Nightingale, carbohydrate/insoluble nitrogen is the causative ratio, while others insist upon the virtues of a starch/nitrogen ratio.

In deciding to give the ratio the true meaning "carbon"/"nitrogen," the carbon embracing all forms of carbon, and nitrogen all forms of that element, many considerations were viewed.

First of all, the primary value of the relation lies in the fact that the growth of the plant is dependent upon the balance between the metabolic processes of carbon assimilation and respiration on one hand, and nitrogen assimilation on the other. Both these processes involve the use of "raw" inorganic material in the form of carbon dioxide and nitrates, and hence to the plant itself the relation of real importance is that of elemental carbon to elemental nitrogen.

Part of the assimilated carbon is of course transformed into immobile compounds such as lignin, incorporated in cell walls, etc., but to say that this is outside the vital system is more supposition than proven fact. We have at present no evidence for or against the theory that this immobile carbon is without influence on the metabolism of the plant.

Again, its very presence there, even as immobile compounds, may constitute a limiting factor to the taking in of further supplies, for as Gurjar has shown, as the carbon increases, photosynthesis automatically decreases. Moreover, it was found in the field that plants starved of mineral salts tend to become very hard and woody showing that the carbon in excess of that balanced by nitrate for vegetative growth does not accumulate as reserve carbohydrate but tends to be further condensed and used for extra lignification, a fact which points to some internal relation between mobile and immobile compound formation, dependent upon the total absolute supplies of carbon and nitrogen.

Lastly, the impossibility of any accurate estimation of small quantities of sugars, starch, or polysaccharides and the possibility of omitting other mobile forms of carbon present, in such parts of the plant as the embryo, the stamens or the ovules, caused the final adoption of the significance of total carbon/total nitrogen for the ratio. The fact that results showing a definite trend were obtained in all cases under consideration, afterwards justified this decision.

Considering Palladin's<sup>(40)</sup> theory that respiration depends upon the amount of nuclein nitrogen present in the cell, the similar conclusion of Nightingale<sup>(42)</sup> that the decisive ratio was that of carbohydrate/insoluble nitrogen and the consideration of Stiles<sup>(55)</sup> that inorganic nitrogen is outside the living system, it was at first thought desirable to estimate nitrate and nitrite nitrogen separately. The Devada's alloy method has been shown to be inapplicable in the presence of asparagin and Strowd's<sup>(54)</sup> method of comparison is equally inaccurate when ammonia or amide nitrogen enter into the system.

Burrell and Phillips<sup>(5)</sup> used an original colorimetric method modified from the pheno-disulphonic acid method, but this was very difficult to manipulate and almost impossible with quantities less than one gram in weight.

A modification of Crum's nitrometer method was next devised, using a micronitrometer measuring quantities of gas under 1 c.c. Comparable results were certainly obtained by this method, bu

inaccuracies were rife owing to the presence of amides, etc. in the plant extract. On account of unavoidable inaccuracy with small amounts of material (and in the light of the decisive values of the  $C/N$  (*total*) found from later results), it was deemed advisable to abandon the idea of a separate estimation of organic and inorganic nitrogen and to concentrate upon a method which would measure accurately the total nitrogen.

Three methods are applicable in general to the estimation of total nitrogen (Association of Official Agricultural Chemists) (1):

- (1) Kjeldahl method modified to include Nitrogen of nitrates.
- (2) Gunning method modified to include Nitrogen of nitrates.
- (3) Absolute or cupric oxide method.

In a decision as to the relative merits of these methods, another important factor had to be considered. That was the amount of available material. In order to rule out genetic variability, pure line seeds from one ear only were used, thus strictly limiting the material to about 40-50 seeds. Assuming that ten sets of estimations only are made, one is thus limited to one plant per estimation. The average dry weight of the embryo is 0.0005 gm., and not until well over the seedling stage does the weight reach 0.1 gm. For this cause, the apparatus finally decided upon was the "Micro-Dumas" apparatus designed by Fritz Pregl ((47) p. 96).

The accuracy of this apparatus for all forms of nitrogen, nitrate, nitrite, and organic nitrogen was tested and the following errors were noted:

Urea: Calculated percentage N 46.66 per cent. Estimated 46.8 and 46.00 per cent.

Potassium nitrate: Calculated 13.8 per cent. Estimated 13.97 per cent.

Sodium nitrite: Calculated 20.29 per cent. Estimated 19.40 per cent.

Mixture of above three substances: Calculated 0.0100 gm. Estimated 0.0098 gm.

By this method the percentage nitrogen on the dry weight basis was obtained.

The carbon was estimated by a modification of the usual combustion apparatus designed by Pregl ((47), p. 23). A few slight modifications were made to suit the conditions under which the work was carried out.

The process consists essentially of the conduction of dried and carbon dioxide free air or oxygen over a suitably filled combustion

tube and absorbing the products of oxidation, i.e. water and carbon dioxide in calcium chloride and soda lime tubes.

In his method Pregl(47) uses liquid air as a source of oxygen but owing to the amount of work to be done and the impossibility of keeping liquid air for any length of time, the oxygen used was from a cylinder of the compressed gas. An amount of this gas sufficient to fill a large jar was collected by the displacement of water, thus being stored at atmospheric pressure. However, as experiments continued and oxygen was withdrawn, reduced pressure was created in the oxygen container, thus causing a backward pull of air. This difficulty was overcome by attaching to the outlet lever of the Marriott flask a three way tap and extending one arm so that it was sealed in the oxygen container. Thus, while air was passed over the apparatus, the water was allowed to drip into the measuring cylinder as in the Pregl method, but when the supply of oxygen was turned on, the tap on the lever arm was turned, sending the water down into the oxygen container. Thus each c.c. of oxygen was replaced by 1 c.c. of water so keeping the gas at atmospheric pressure.

Pregl, in his method, passes the air or oxygen into the combustion tube through a tapering capillary tube fitted in by means of a cork. This was considered unsatisfactory for two reasons. First, the inability to procure and maintain perfectly fitting corks, and secondly that the hot air inside the tube is liable to decompose the rubber of a cork, setting free highly carbonaceous products and impairing the accuracy of the estimation. The following method was adopted, using instead of a capillary tube a stopper from an absorption tube joined to the combustion tube with strong pressure tubing. This had two advantages, namely that the gas entered the tube and left it through exactly similarly bored and constricted tubes and secondly that should any heating of the rubber connection take place the products of its decomposition would be outside and not inside the system. Care was taken that the two glass edges were always in contact (Fig. 2). The combustion tube was filled as in Fig. 3; no alteration being made from Pregl's method.

A slight modification of the soda lime absorption tube was found necessary in that the amount of calcium chloride was reduced until it was found that best results were obtained when it was completely eliminated and the whole tube was filled with very fine granular soda-lime, slightly moistened. It was necessary to refill the absorption tubes after every third experiment.

After the third absorption tube an innovation in the form of a second bubble counter was placed in the system. This enabled me to determine with accuracy if any leakage was occurring in the circuit, since the bubble frequency in each counter should be the same.

For convenience in refilling, an aspirator fitted with a tap for air outlet, was used as a Marriott flask.

All weighing was performed upon a Pregl-Oertling micro-balance, which enables readings to be made to 0.010 mgrm.

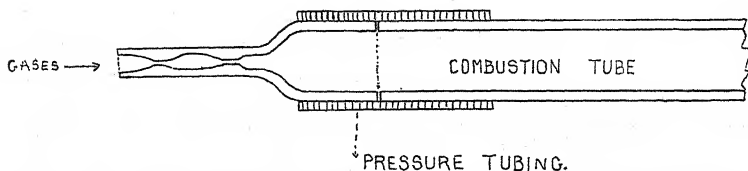


Fig. 2. Junction of stopper from an absorption tube with the combustion tube.

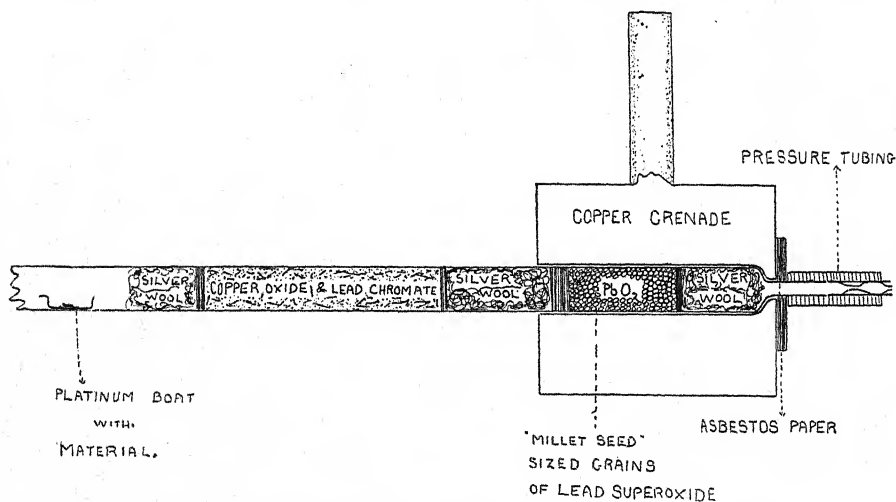


Fig. 3. Pregl's apparatus as employed in the experiments described.

## RESULTS

In order to avoid unnecessary repetition or complication, it will be advantageous to consider each strain separately.

*"Starling" Winter wheat, sown Dec. 25th, 1925.*

For convenience in comparison the growing period of the plant was divided into three cycles: (1) the seedling stage up to the active

assimilation of the first leaf and the exhaustion of the seed's store of food; (2) the vegetative cycle up to ear formation; (3) the fruiting stage.

These agree with the divisions made by Davidson and Leclerc<sup>(10)</sup> for wheat, i.e. (1) 2 in. high; (2) time of heading; (3) milk stage; but differ from those of Burd<sup>(4)</sup>, who divides the life cycle of a plant thus: (1) 6 to 8 weeks, period of increase in dry weight; (2) 6 weeks, drop in dry weight, increase up to head formation; (3) loss in weight of whole plant.

However arbitrary these divisions seem at first it will be seen by the results that they represent distinct metabolic phases and also the first two of Burd's<sup>(4)</sup> divisions will make a natural subdivision of my vegetative cycle.

The seedling stage was considered in detail and estimations were made on the fourth, seventh, tenth, and fourteenth days. During the vegetative phase, the stages selected were: first leaf, third leaf, fifth leaf, tenth leaf and ear initiation, while during the fruiting stage attention was paid chiefly to the changes in the ear itself, the developing ovary and embryos, although the vegetative organs were not neglected.

During the seedling stage the whole plant was used for each estimation, and plants as nearly as possible morphologically similar and from seeds of similar weight were chosen. The material was re-dried to constant weight and estimated by the methods described above.

As the plants developed it became impossible to estimate the whole plant so that it was first of all divided into its component parts of root and plumule. The plumule was often bisected longitudinally, the halves being used for duplicate estimations. During the entire vegetative period the root, stem and leaf were estimated separately, and where these were too large even for the estimation of narrow longitudinal strips, the parts were ground to a very fine powder and aliquot samples taken. The results were expressed as percentage dry weight, as allowing the best basis for comparison, and in every case at least duplicate estimations were made. Where discrepancies between duplicate results were outside the limits of experimental error, a fresh pair of estimations were made.

After the first seven or eight weeks it was evident that all plants would not reach the same stage of development at the same time; but whether the carbon and nitrogen content depended upon actual age or developmental condition was unknown. To settle this question,

on the 55th day two plants were taken, one of which had just developed its fifth leaf and had a single tiller, and a second which had the fifth leaf fully developed, the sixth leaf appearing and two tillers. Each was longitudinally divided and the internal conditions of C/N investigated. Results marked 1 and 2 in Table I show clearly that the carbon and nitrogen content follows the development of the plant irrespective of its age in days.

All plants were vigorously vegetative and strikingly healthy. Each had 2-4 tillers and the leaves were long, broad and of a full green. The next step was shown at the tenth leaf stage where dissection showed as yet no trace of ear initiation. This seems to have occurred just after the development of the twelfth leaf judging from the majority of plants. At that time a general estimation of the vegetative parts of the plant by powdering was made, but attention was transferred to the ear as representing the chief seat of changes during the third or fruiting cycle. Changes in the chemical composition of the flowers were traced, and the sexual organs were investigated during their development, especially the ovaries up to the mature embryo. The full numerical results are shown in Table I, and a graphical representation is made of the outstanding features in this table in Figs. 4, 5 and 6.

*Interpretation of data.* As regards the interpretation of the results, I should like to point out at the beginning that little stress is laid upon the actual figures, since they depend to a large extent upon external conditions affecting the metabolic processes of respiration, photosynthesis and absorption, which were outside control in this experiment. Gregory (19) has shown us that external factors work either separately or in groups in control of such processes as assimilation, yet the process of dry weight increase is not controlled solely by outside influences. This result is readily explicable in the light of the present results, in that dry weight, chiefly the result of carbon accumulation, depends not only upon the assimilation and respiration relation, but upon the amount of available nitrogen to balance it and use it for growth.

However since here we have a series of analytical results showing a marked tendency in one direction, we are justified in accepting them as criteria of metabolic conditions. I am convinced that they will be found representative not only of this strain of wheat but of other annual plants of similar growth.

The seedling stage is characterised by a rapid drop in the carbon and the nitrogen percentage. Carbon is actually lost by respiration



# The Carbon/Nitrogen Ratio in the Wheat Plant 21

TABLE I.  
*Showing results of analysis of the different organs at various ages of the winter wheat "Starling."*

Age	Plant organ	Carbon (1) % dry wt.	Carbon (2) % dry wt.	Carbon Average % dry wt.	Nitrogen (1) % dry wt.	Nitrogen (2) % dry wt.	Nitrogen Average % dry wt.	C/N root	C/N stem	C/N leaves	C/N other parts
Seed	Endosperm	30.303	31.031	30.967	2.923	2.901	2.912				
	Embryo	50.249	50.505	50.377	13.314	13.447	13.382		Whole plant 3.8		Endosperm 10.6
4 days	Embryo	47.752	46.3636	47.055	12.232	12.280	12.241		Whole plant 3.8		
7 days	Embryo	34.0909	34.615	34.353	8.792	8.956	8.874		Whole plant 3.8		
10 days	Plumule	27.27	30.909	29.089	7.042	7.804	7.423			Plumule 3.9	
	Radicle	38.0363	40.043	39.339	3.053	3.1452	3.099	12.7			
14 days	Plumule	28.20	31.818	30.009	7.318	7.108	7.213				
	Radicle	38.352	40.660	39.507	3.152	3.0331	3.092				
33 days, Third leaf stage	Stem	35.832	33.471	34.654	5.962	5.760	5.861	12.8	4.16		
	Radicle	38.801	37.76	38.1825	3.105	2.952	3.0285				
	Leaves:										
	First and second	35.201	34.27	34.9261	6.7799	6.904	6.7745	12.6	5.9	5.1	
	Third	36.0858	34.101		6.765	6.654					
1, 55 days, Fifth leaf stage	Stem:										
	Main shoot	35.839	—	35.839	4.706	—	4.706				
	Secondary shoot	38.815	—	38.815	5.064	—	5.064				
	Radicle	36.98	38.961	37.97	3.376	3.426	3.401				Sideshoot (1) 7.7
	Leaves:										
	First	35.201	34.27	34.9261	6.7799	6.904	6.7745	11.1	7.0	6.2	
	Second	36.086	34.101	35.592	6.7650	6.654	6.710				
	Third	38.149	38.035	38.092	4.8070	5.000	4.9035				
	Fourth	41.624	41.565	41.5945	5.255	—	5.255				
	Fifth	38.385	—	38.385	5.392	5.305	5.3485				
2, 55 days, Sixth leaf stage	Stem:										
	Main shoot	35.739	—	35.739	4.416	—	4.416				
	Secondary shoot	38.961	38.670	38.815	5.064	—	5.064				Sideshoot (1) 7.7
	Second sec. shoot	44.724	—	44.724	5.906	—	5.906				
	Radicle	36.98	38.961	37.97	3.426	3.376	3.401	11.1	8.1	6.7	
	Leaves:										
	First to fifth	37.502	—	37.502	6.021	—	6.021				Sideshoot (2) 7.45
	Sixth	38.085	—	38.085	5.255	—	5.255				

TABLE I (continued).

Age	Tenth leaf stage	Plant organ	Carbon (1) % dry wt.	Carbon (2) % dry wt.	Carbon Average % dry wt.	Nitrogen (1) % dry wt.	Nitrogen (2) % dry wt.	Nitrogen Average % dry wt.	C/N root	C/N stem	C/N leaves	C/N other parts
Tenth leaf stage	Stem:	Main shoot	38.034	(Average)	38.034	2.950	(Average)	2.950	17.1	13.0	7.6	
		Secondary shoot	39.362	37.97	38.669	—	—	—				
		Radicle	37.391	37.121	37.255	2.111	2.243	2.1707				
		Leaves:										
		Tenth:	30.46	31.302	28.856	3.764	4.741	3.807				
Ear stage	First to ninth	Stem	26.91	26.70		3.377	(Upper half)		22.3	31	17.8	Ear unemerged 13.5 Ear emerged 17
		Root	35.29	37.001	36.145	1.308	1.020	1.164				
		Leaves	35.896	35.604	35.700	1.606	—	1.6066				
		Ear (unemerged)	36.466	35.761	36.114	1.846	2.206	2.026				
		Ear (emerged)	35.139	35.796	35.467	2.668	2.599	2.634				
Ripe fruit stage	Stem:	Stem	38.817	38.621	38.719	2.367	2.212	2.289	24.3	22.6	17.1	Ovaries 15.1 15.9 16.7
		Main shoot	(Comp. av.)	31.4	31.4	(Average)	1.390	1.390				
		Secondary shoot	"	32.44	32.44	"	1.5625	1.5625				
		Root	"	31.666	31.666	"	1.294	1.294				
		Leaves:										
		Main shoot	"	35.937	35.937	"	2.107	2.107				
		Secondary shoot	"	32.602	32.602	"	2.022	2.022				
		Ear (ripe)	42.868	42.723	42.796	2.057	1.9855	2.020				
		Stamens:										
		Unburst (1)	41.7	40.19	40.94	7.385	—	7.385				
		Burst (2)	32.67	31.31	31.990	6.624	—	6.624				
		Ovaries:										
Ripe fruit stage	Stem:	Unfertilised	38.0726	38.646	38.358	2.548	2.547	2.5475	20.8	22.6	17.1	Embryo 5.7
		Fertilised ovary	39.831	39.775	39.813	2.31	—	2.31				
		Ripe seed:										
		Embryo	50.369	49.019	49.694	8.502	8.943	8.723				
		Endosperm	30.7	—	30.7	1.986	2.280	2.180				

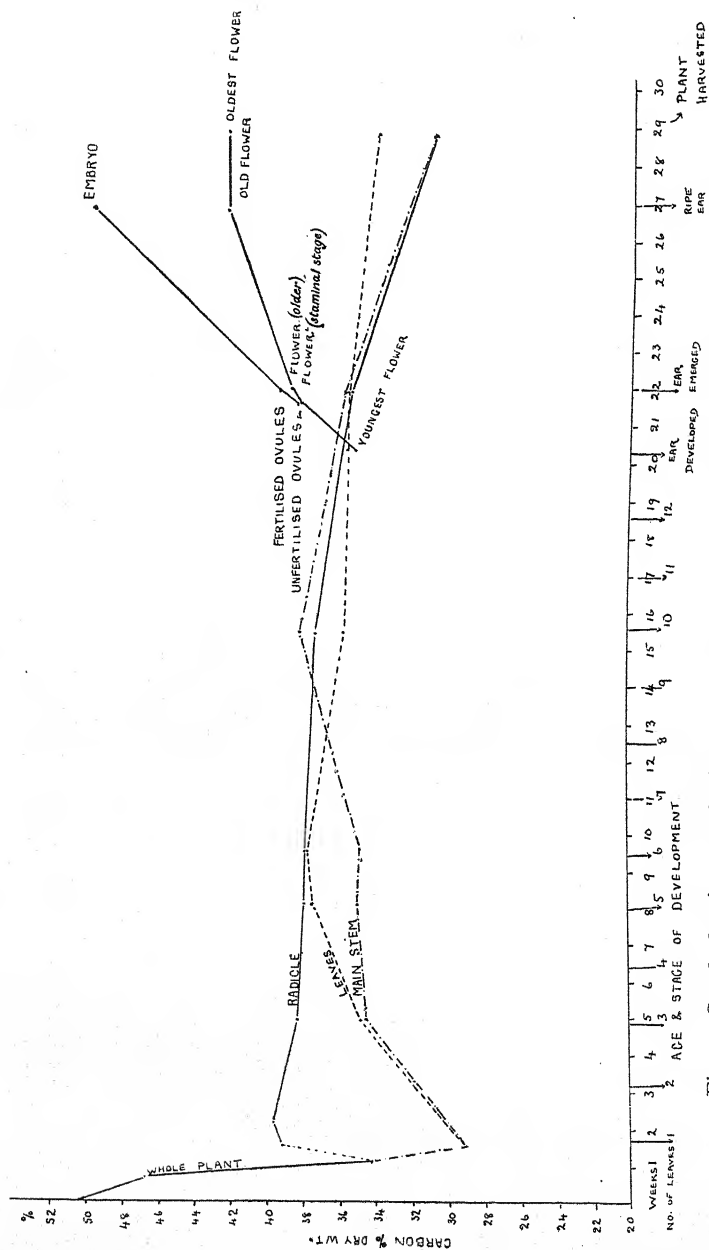


Fig. 4. Graph showing march of carbon percentage of dry weight in "Starling" winter wheat.

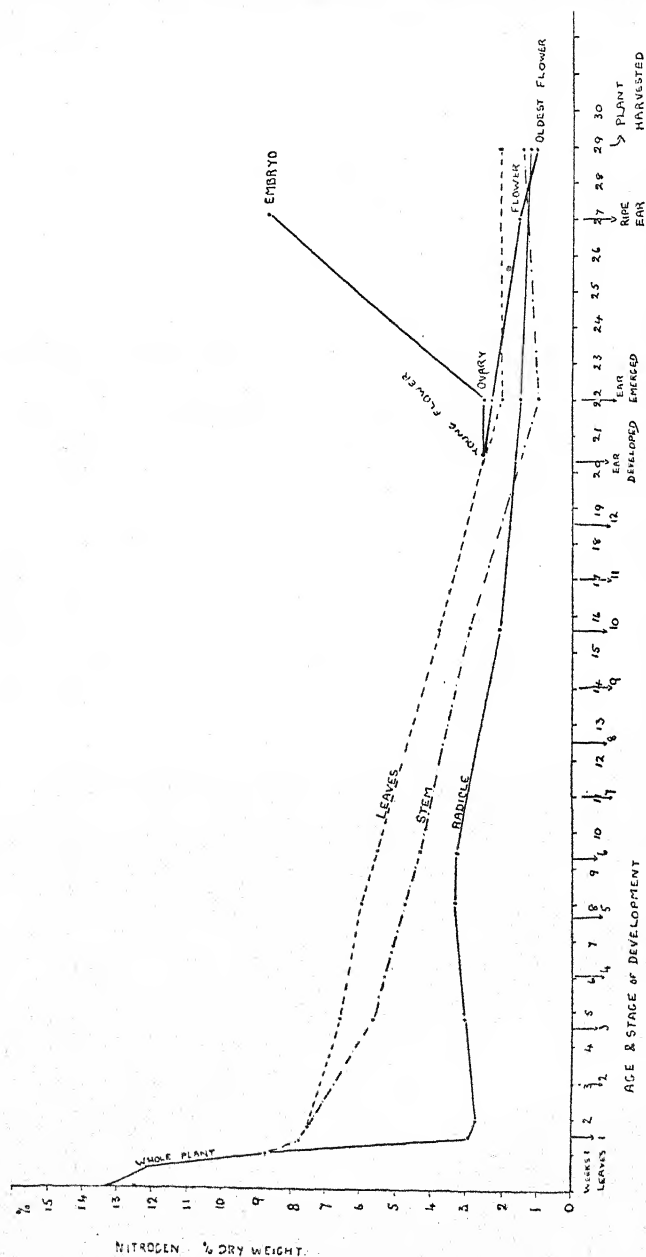


Fig. 5. Graph showing march of nitrogen percentage of dry weight in "Starling" winter wheat.

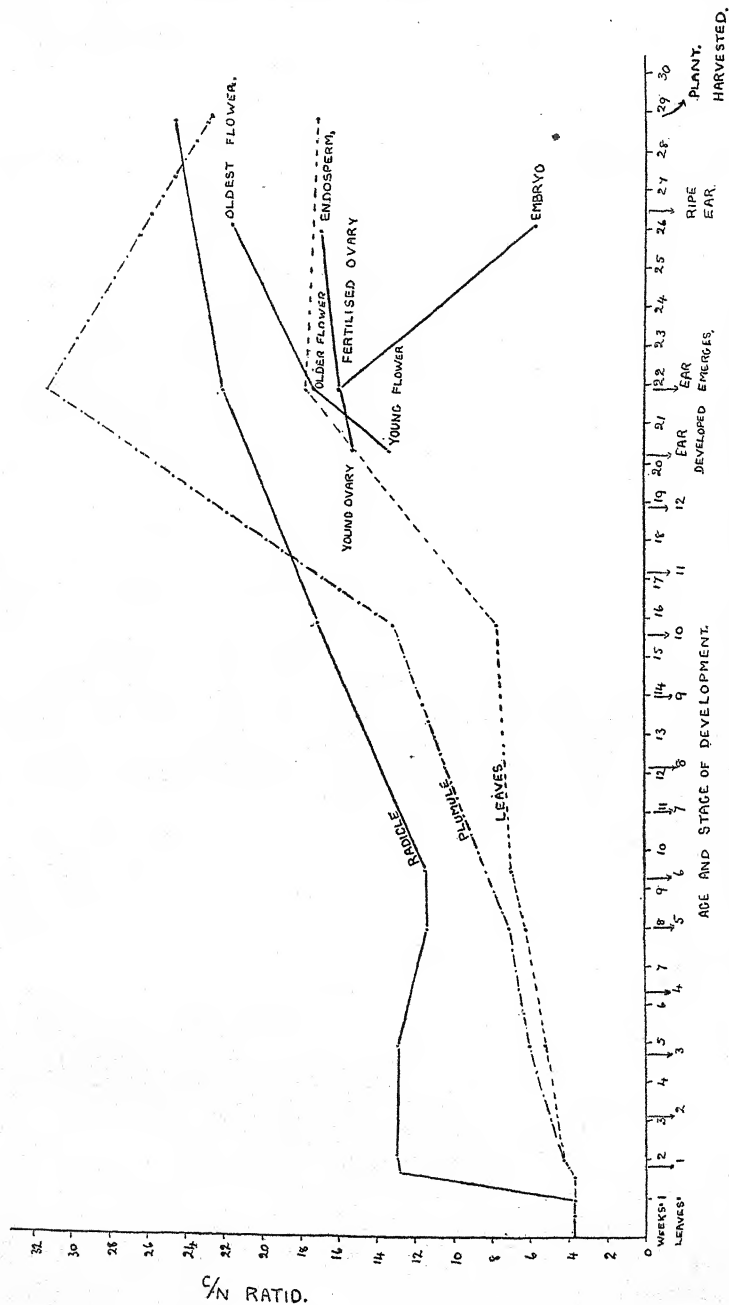


Fig. 6. Graph showing march of C/N ratio in "Starling" winter wheat.

but most of the large reserve in the seed is withdrawn into the embryo, thus increasing the dry weight while decreasing the percentage of that element. No nitrogen is lost, the actual amount is increased by the withdrawing of the reserves from the endosperm, while the percentage is decreased owing to the increased dry weight.

Davidson's experiments (8) have recorded a loss in nitrogen in five days old wheat seedlings grown in distilled water, and then a slight gain upon the seventh day; while Lipman and Taylor (36) on the other hand report large gains in wheat grown without nutrient nitrogen due entirely to nitrogen fixation from the air by the wheat plants. From my own experiments it must be stated that during the seedling stage results of analysis show that there is very little change in the nitrogen content of seed and seedling taken together, and no support can be given either to the nitrogen fixation theory or to the idea of nitrogen loss by leaching.

The percentage loss of carbon and nitrogen is obviously a process internally controlled since during the seedling stage, the C/N ratio is very low, and remains constant, and the same as that of the ungerminated embryo. It is to be noticed that the C/N ratio is very low, due to high nitrogen, since the carbon itself is high. This maintenance of the low embryonic ratio is interesting in view of Palladin's (46) results. He finds that for germinating seeds where carbon is in excess, respiration increases day by day and the carbohydrates steadily decrease. However the essential non-digestible proteins increase *pari passu*, and, assuming these to be a measure of the actual protoplasm, he finds an approximately constant ratio between the amount of protoplasm at any stage and the respiration. This suggests a perfect balance between C and N which the present analyses show to be actually the case.

✓ The vegetative cycle opens with a low carbon and fairly high nitrogen content and a low C/N balance.

This has been supposed by previous workers to characterise vigorous vegetation and non-fruiting. This is borne out by these results for a non-reproductive period of healthy vegetative development sets in. However, immediately after the emergence of the first leaf and the beginning of active photosynthesis, there is a rise in the carbon curve. In this set of results it will be remarked that the rise is rather sudden, during the first three or four weeks, after which there is a slackening of the carbon accumulation. This has its explanation in the fact that for the first four or five weeks the standard dilution of nutrient fluid given in the culture formula was

used for watering, and the plants became woody at the base and slack in development, as a result of starvation, or, in clearer terms, for lack of nitrogen to balance the carbon and assist growth. After that time the dilution was decreased as mentioned before. The curve then showed a gradual rise, having its maximum after 16 weeks or at the tenth leaf stage. It then began to fall gradually. This corresponds to the two classes of Burd(4), who shows that the dry weight increases rapidly up to a maximum, and then the increase falls off until ear formation.

During all this time there is a steady decrease in the percentage of nitrogen in every organ of the plant, the response to the increased culture strength being shown slightly only by the roots, while the curve for the aerial portions approaches a straight line.

The C/N ratio shows a marked increase throughout the whole period. Most of the earlier workers seem to have reached the conclusion that flower initiation is set up as a result of carbon accumulation, and any factor inhibiting or retarding starch accumulation, such as shade or short day, would on this reasoning inhibit or retard flower production. If this were correct, flower primordia should appear at the carbon maximum. This, however, is not the case, for after the maximum carbon content is reached there is a rather rapid fall in carbon before flowers are formed, for it is with the interrelation of carbon and nitrogen that flowering is associated.

✓ Flower initiation is not determined by a high carbon content but by a high C/N ratio.

These results agree with the curves of Gilbert(18) for *Amaranthus* but differ from those of Woo(59), for the same plant, since the nitrogen curve shows no minimum corresponding to the carbon maximum. Woo's curves for root, stem and leaf are of the form shown in Fig. 7, where it is obvious that the C/N ratio falls towards the end of the growth period and flowering, due to increased nitrate.

Campbell(6), however, has disproved the presence of nitrate in *Amaranthus*, at the time of flowering, which would thus probably bring the results for that plant into line with those of wheat.

At the epoch of highest C/N ratio, flower initials appear, and there begins one of the most important features in fruit formation, namely a general movement in the plant of the carbohydrates and the soluble nitrogen constituents through the stem and into the reproductive parts. The stamens and young ovaries are both rich in carbon, although the stamens lose carbon as the pollen is shed. The fertilised ovaries continue to pile up carbohydrates in the form

of starch, while the nitrogen increase is chiefly localised in the ovule itself.

During this time the C/N ratio decreases in the developing organs, until a very low value coupled, however, with high carbon and high nitrogen is exhibited by the mature embryo.

No better summary of the facts could be stated than Kraus and Kraybill's own words "Fruitfulness is associated neither with the highest nitrates, nor the highest carbohydrates, but with a condition of balance between them."

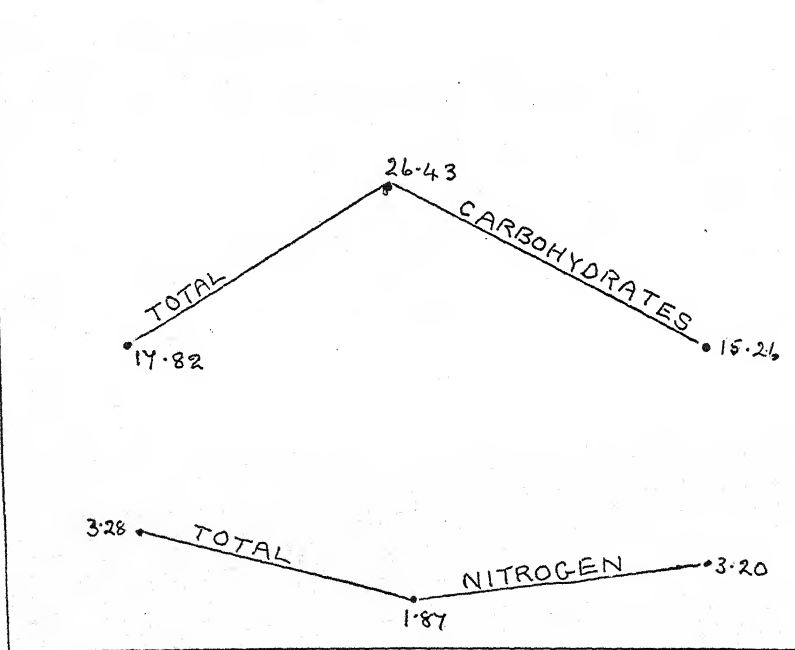


Fig. 7. Reciprocal fluctuation of carbohydrates in roots (Woo).

It also brings out the fact that conditions of fruit set are different from those of flowering since flowering is characterised by a high C/N ratio with depleted nitrogen, while in order to produce fertile seeds a rich store of carbon and nitrogen is required, resulting in a low C/N ratio.

During the fruiting cycle growth in the vegetative organs has been at a standstill. There is a slight loss in carbon and a cessation of nitrogen absorption and morphologically senescence is very obvious. Nitrogen flows from the gradually dying leaves but as



Fig. 5 and Table I show the translocation is upwards towards the ear and not downwards towards the root.

By this one complete life cycle at least three of Kraus and Kraybill's<sup>(32)</sup> four assumed classes are shown to be valid, i.e.:

Class 2. Abundant nitrogen and moderate carbon which give vigorous vegetation and sterility is shown by conditions at the beginning of the vegetative stage.

Class 3. Decrease in nitrogen and an increase in carbon, yielding less vegetation and increased fruitfulness—shown by the conditions at the end of the vegetative stage.

Class 4. Still less nitrogen and the same carbon—suppression of vegetation and reproduction—demonstrated by the senescent vegetative organs after flowering.

It must be noted here that the nitrogen of the ripened embryo was much less than in the original embryo. Garner, Allard and Foubert<sup>(16)</sup> state that seeds as a rule are constant in composition and true to maternal type although they are sometimes very greatly affected by their conditions of cultivation. This is probably the case here since the carbon, which was outside control (i.e. atmospheric  $\text{CO}_2$ ) was the same, but the plants were artificially fed with nitrate, which judged by this test was apparently inadequate.

Another question was suggested by Kidd, West and Briggs<sup>(35)</sup> who point out that the initial respiratory index of successive leaves, i.e. the respiratory index of the stem apex, decreases with the age of the plant, indicating a progressive decrease with age in the rate of respiration in meristematic tissue. Their work was done on *Helianthus* but it was thought quite probable that the C/N relations would also vary with age in meristematic tissue of wheat if respiration varied. Thus the first six leaves of the developing Starling plants were estimated for C/N conditions at a similar time just after their unfolding. The blade of the leaf was cut in half and estimated as "tip" and "base" but the sheathing base of the leaf was not examined as it could not be removed without injury to the plant.

Table II shows clearly that the initial carbon in successive leaves increases with the age of the plant, the nitrogen decreases and the C/N increases.

A similar state of affairs is shown by the developing tillers, which contain on initiation a carbon and nitrogen content slightly higher than that of the leaf near whose base they arise, and the later they arise the higher their initial C/N ratio, following the rule for the leaf.

TABLE II.

*Carbon and Nitrogen content of leaves as influenced by time of development.*

		C (1)	C (2)	Average	N (1)	N (2)	Average	C/N
First leaf	Tip	35.45	39.01	37.23	6.102	6.521	6.311	6.0
	Base	33.09	31.49	32.29	7.288	7.457	7.37	4.7
Second leaf	Tip	36.36	35.45	35.90	7.22	6.769	6.99	5.1
	Base	35.808	32.74	34.27	6.76	6.086	6.423	5.3
Third leaf	Tip	37.169	40.90	39.03	5.97	6.18	6.07	6.5
	Base	36.36	39.13	37.74	4.71	4.83	4.77	7.8
Fourth leaf	Tip	39.312	39.958	39.635	5.162	5.133	5.147	7.7
	Base	43.936	43.173	43.56	4.48	4.83	4.65	9.3
Fifth leaf	Tip	38.961	38.961	38.961	4.88	5.216	5.047	7.8
	Base	38.689	—	38.689	5.01	—	5.01	7.7

*"Nevin Bearded" English spring wheat.*

"Nevin Bearded" was the next set to be planted. The seeds were sown on March 23rd. The methods of germination were the same as for Starling—in distilled water until the tenth day and then in sand, watered with the same culture solution, at the same time and under the same physical conditions.

The seedling developed much more rapidly than did the Starling wheat and in three days had developed to the same stage as four-day Starling and in six days to the seven-day Starling stage. Estimations were therefore made during the seedling stage at the same time periods in days and the same growth stages. During the vegetative phase it seemed unnecessary to make estimations at both the same time and growth periods, since so little difference occurred between them in actual development, i.e. the third leaf developed in Starling on the 33rd day, and in Nevin after 28 days, while on the 33rd day very little advancement had been made from that of the 28th day. Thus only similar growth stages were taken, i.e. third leaf stage, fifth leaf stage and sixth leaf or ear stage.

The chief difference between the two strains is the much longer period of vegetative activity in the winter wheat, in which 12 leaves develop before ear formation, whereas in the spring wheat, flowers appeared after the unfolding of the sixth leaf, after 76 days. This wheat had the stronger culture solution throughout its entire growing period but after transfer to sand, the plants grew very slowly and appeared unhealthy. However, later they recovered rapidly as they became accustomed to the greenhouse conditions and were quite sturdy plants by flowering time. They tillered much

less however than the Starling, that plant showing 6-8 tillers while 2 was the average for this variety.

Table III gives the numerical results of the analyses on a dry weight basis. Figs. 8, 9 and 10 are graphs showing the outstanding features.

The C/N relations in this strain are strikingly similar to those of Starling. The seedling stage is characterised by the same rapid drop in carbon and nitrogen and the constancy of the C/N ratio.

After the carbon minimum is reached on the seventh day there is a very rapid rise in the carbon content, during the time when the plant appeared unhealthy, an obvious example of Kraus and Kraybill's (32) Class 4 with high carbon and below medium nitrogen giving little vegetation and no fruit. The carbon maximum was reached after 14 days, that in Starling after 16 weeks, thus accounting for the difference in length of vegetative period. After 14 days a rapid falling off of carbon occurred; the nitrogen fell off gradually and the C/N continued to rise until a value of 16.8 in the stem was reached at which flower primordia formation was favoured. This has to be compared with a ratio of 31 in Starling stem at that time.

After flower production all "developing" organs showed a distinct gain in carbon and nitrogen with a decrease in C/N. The original low value of the C/N was, however, never reached, the young embryo being less rich in nitrogen than its parent. A more detailed comparison of the strains will be given later.

*"Marquis"—an American spring wheat.*

This wheat was sown a month later than "Nevin" and reached maturity little more than a week later than that strain.

It was distinguished by its very rapid germination, the first leaf developing in seven days. It was, however, kept in distilled water until the tenth day when it was showing signs of obvious starvation. It was hoped that some of the metabolic results of this starvation would be shown by a deviation from the more normal type of C/N relation. On transfer to sand culture, recovery was complete, no set back being experienced in response to the warm greenhouse conditions, but the plants were always poorly vegetative. Tillering was slow and incomplete, many plants being without a single shoot. The third leaf stage was reached after 25 days, the fifth leaf stage after 45 days, the ear appearing on the 66th day.

Graphical representation of the results of analyses (Figs. 11, 12 and 13) show the same balanced drop of carbon and nitrogen and the

TABLE III.

*Showing results of the analysis of the different organs at various ages of the English spring wheat "Nevin Bearded."*

Age	Plant organ	Carbon (1) % dry wt.	Carbon (2) % dry wt.	Carbon Average % dry wt.	Nitrogen (1) % dry wt.	Nitrogen (2) % dry wt.	Nitrogen Average % dry wt.	C/N root	C/N stem	C/N leaves	C/N other parts
Seed	Embryo	41.322	—	41.322	10.4545	10.58123	10.5134	Whole plant 3.99			
3 days	Embryo	40.205	40.919	40.562	10.214	10.1959	10.2049	Whole plant 3.99			
4 days	Embryo	39.84	39.44	39.64	9.959	—	9.959	Whole plant 3.99			
6 days	Embryo	32.668	—	32.668	7.734	6.994	7.364	Whole plant 4.4			
7 days	Plumule Radicle	29.049 34.449	28.562 34.283	28.805 34.366	6.240 3.550	6.154 3.934	6.197 3.742	Plumule 4.65			
10 days	Plumule Radicle	38.2009 32.727	38.39 31.424	38.3405 32.029	5.952 3.7379	—	5.932 3.6115	Plumule 6.4			
14 days	Plumule Radicle	42.299 35.029	42.56 —	42.429 35.029	4.894 3.461	—	4.894 3.461	Plumule 8.6			
27 days. Third leaf stage	Stem Radicle Leaves	36.837 36.810 39.430	36.909 — 40.155	36.873 36.810 39.792	4.9188 2.566 5.019	4.442 — 4.72088	4.680 2.566 4.869	8.1			
50 days. Fifth leaf stage	Stem Radicle Fifth leaf Leaves 1-4	31.111 29.457 34.362 35.397	31.066 32.308 34.710 34.906	31.088 30.883 34.536 35.152	3.178 2.054 3.40 4.951	2.891 — — —	3.034 2.054 3.40 4.251	15			
76 days. Ear stage	Stem Radicle Leaves Side shoots Ear (70 days)	24.204 29.661 36.409 33.732	24.110 29.509 31.900 —	24.157 29.585 32.0045 30.707	1.403 1.612 3.438 2.381	1.426 2.067 3.5112 —	1.444 1.884 3.5112 2.381	16.8			
Ear (76 days)	Ovaries	33.009	33.009	33.009	2.227	2.236	2.231	—			
Ear (80 days)	Embryo	38.329	38.711	38.520	5.483	5.495	5.489	—			
								Side shoot 20			
								Ear 14.1			
								Ear 14.7			
								7.0			

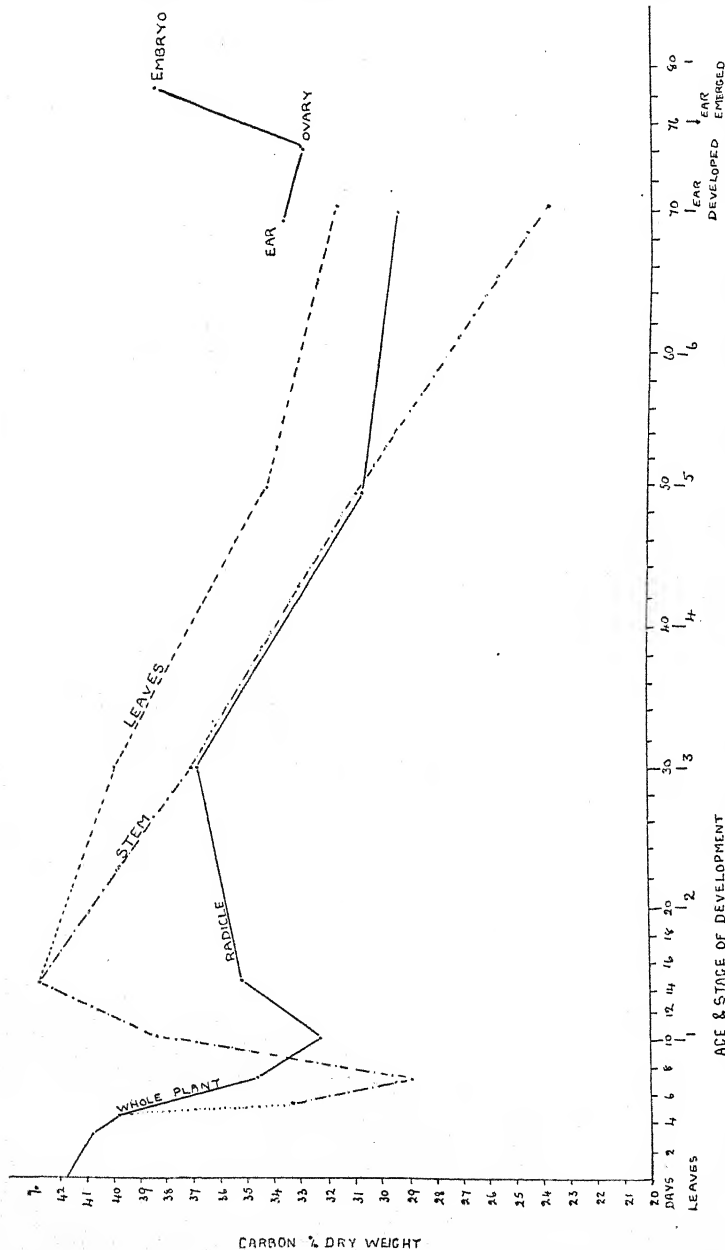


Fig. 8. Graph showing march of carbon percentage of dry weight in "Nevin Bearded" (English spring wheat).

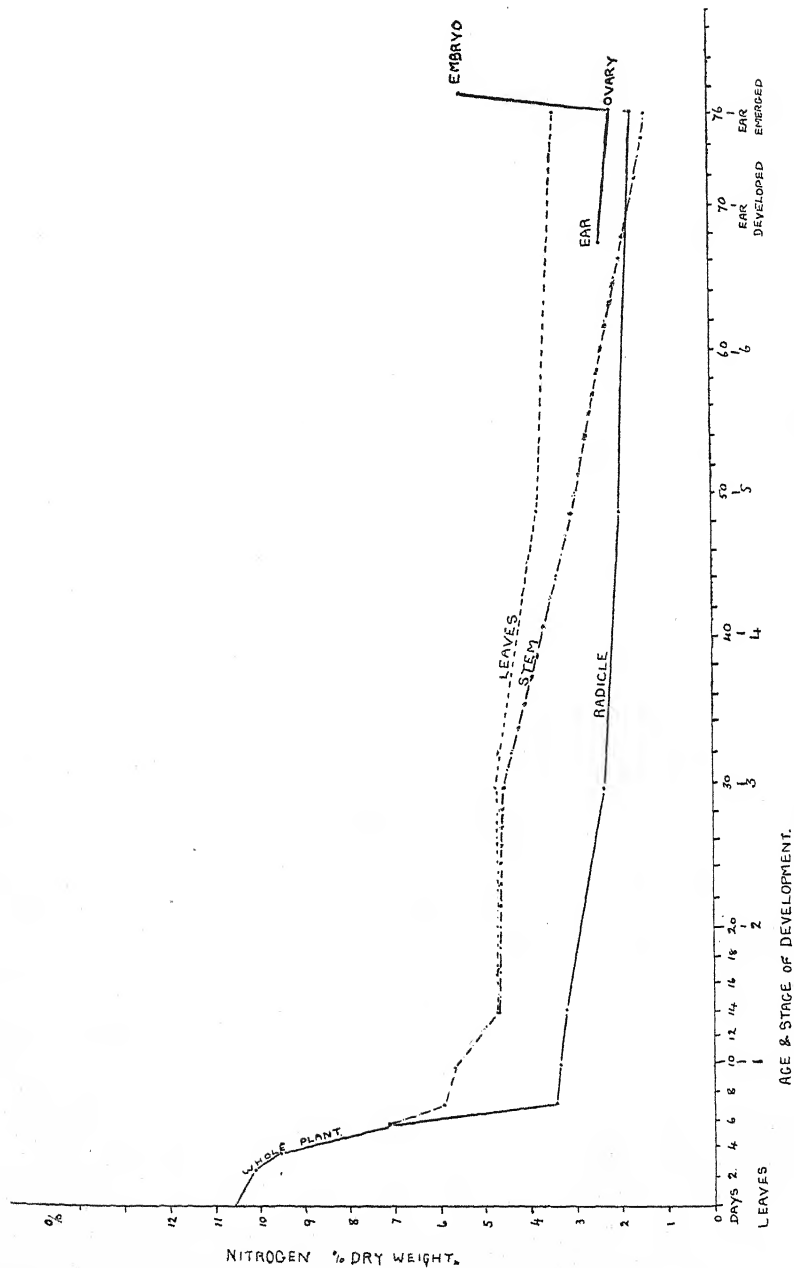


Fig. 9. Graph showing march of nitrogen percentage of dry weight in "Nevin Bearded" (English spring wheat).

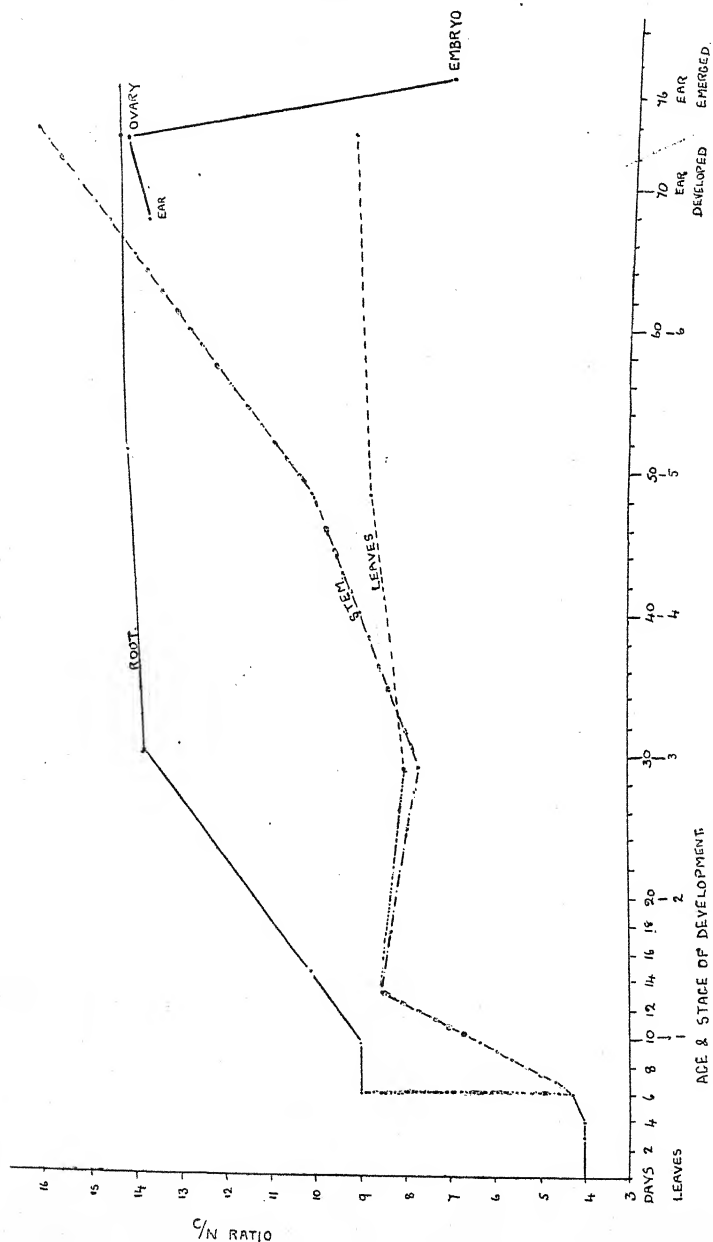


Fig. 10. Graph showing march of C/N ratio in "Nevin Bearded" (English spring wheat).

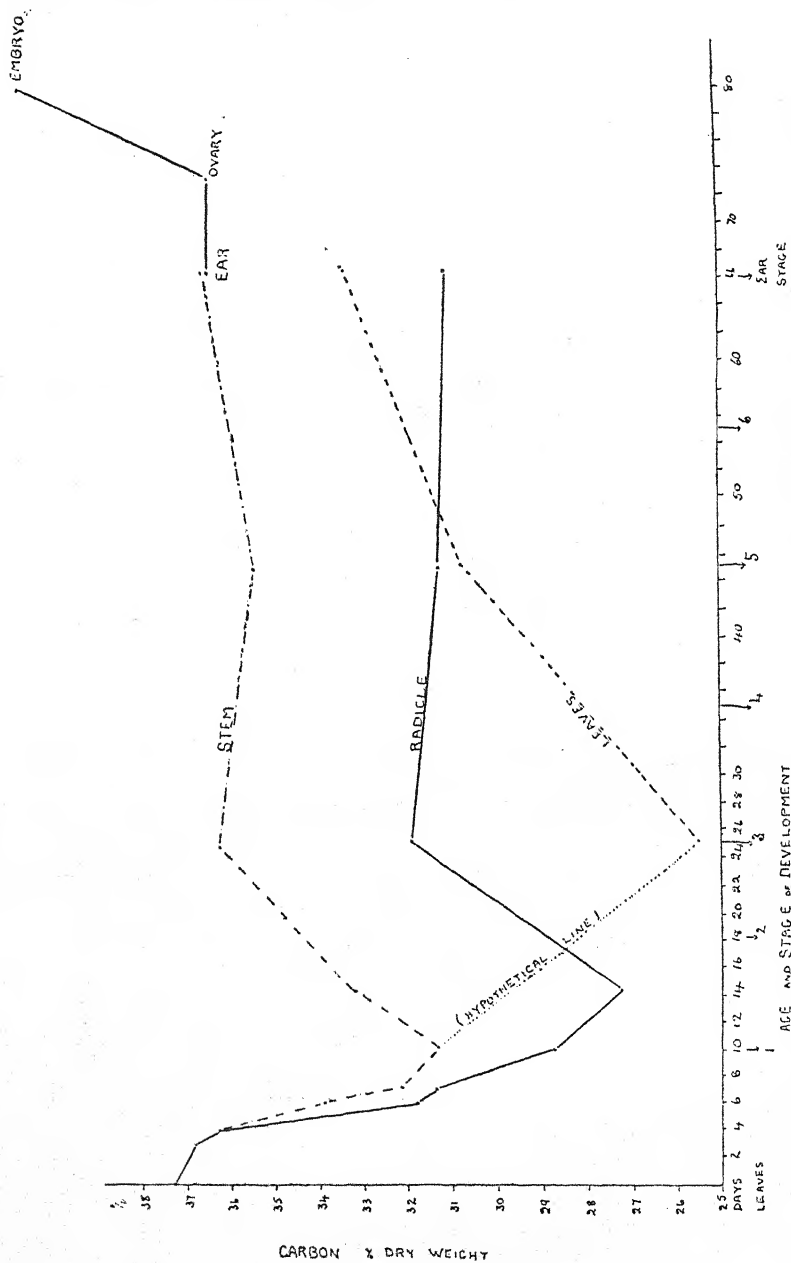


Fig. 11. Graph showing march of carbon percentage of dry weight in "Marquis" (an American spring wheat).



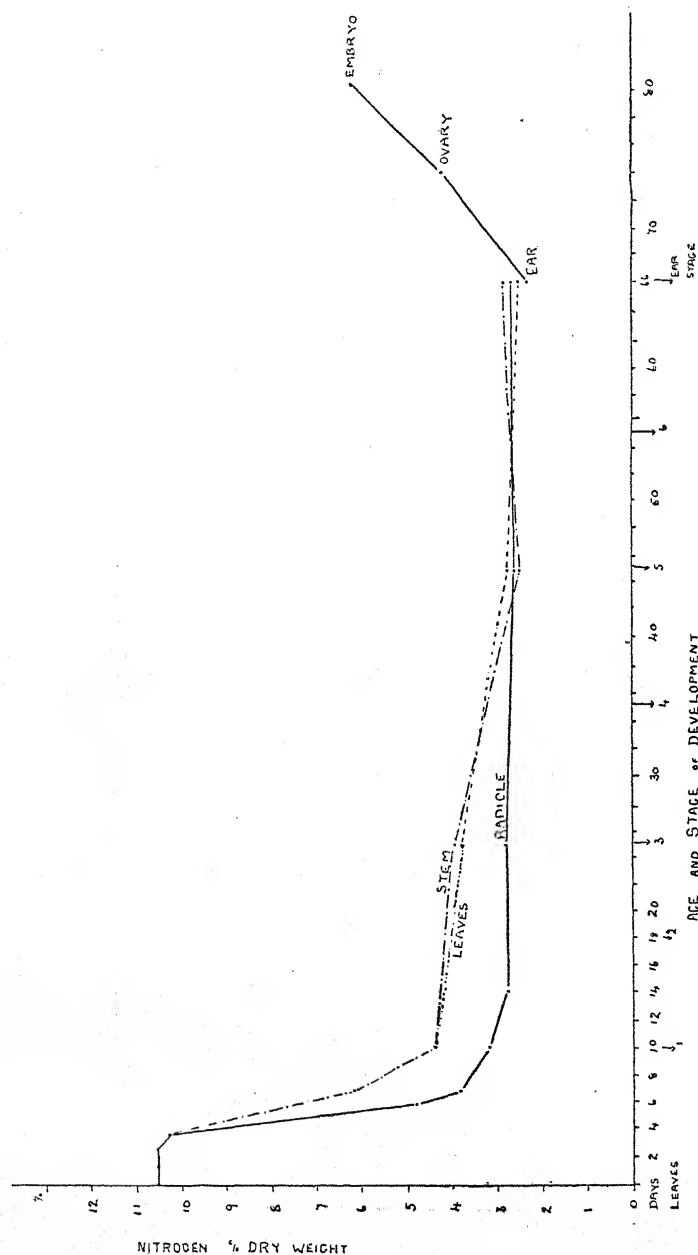


Fig. 12. Graph showing march of nitrogen percentage of dry weight in "Marquis" (an American spring wheat).

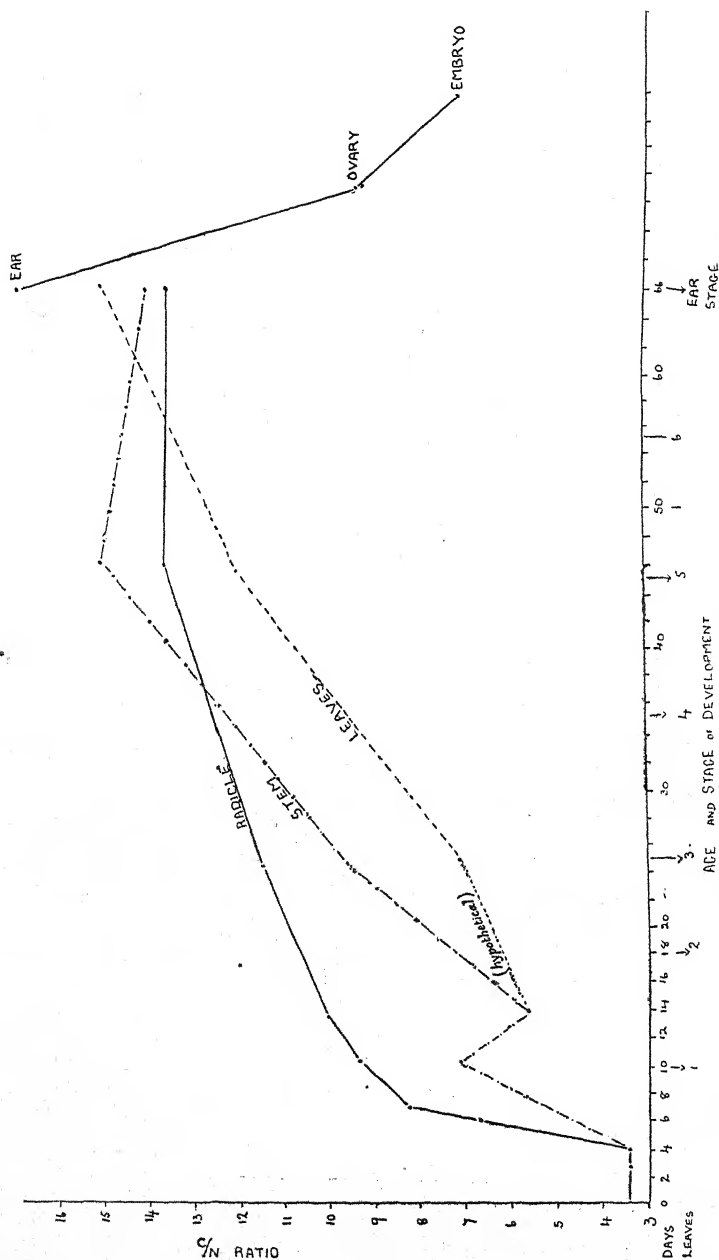


Fig. 13. Graph showing march of C/N ratio in "Marquis" (an American spring wheat).

maintenance of the embryonic C/N ratio during the first stages of germination. However, instead of a rise in carbon, and a continued drop in nitrogen, respiration is evidently still in excess on the tenth day, while the nitrogen percentage is very low. Thus a comparatively high C/N is reached and the plant becomes metabolically prematurely senescent.

Addition of nitrogen changed all this. The nitrogen content of stem and leaves began to rise, while the carbon continued to drop, hence lowering the C/N ratio, until in four days the plant becomes chemically "younger" than hitherto. It then begins its vegetative development with a moderate C/N and normal metabolic changes occur. The carbon rises, reaching its maximum at 25 days, the expected time by comparison with Starling curves, while the nitrogen falls steadily. When the C/N has risen to 15 the ear is formed, and the carbon and nitrogen passes from the plumule into the developing sexual organs as before.

The embryos again show the nitrogen deficiency whereas their carbon content is higher than originally.

#### COMPARISON OF THE THREE STRAINS

The appearance of the three strains may be gathered from the photographs on Plate I. Most interesting conclusions may be drawn from a comparison of the data obtained.

First the striking uniformity in general trend of the three sets of curves for root, stem and leaves must be emphasised; for while the actual figures vary considerably, similar growth conditions are characterised by like metabolic conditions, and it is possible that comparable C/N curves could be obtained for any annual plant with the same kind of life history.

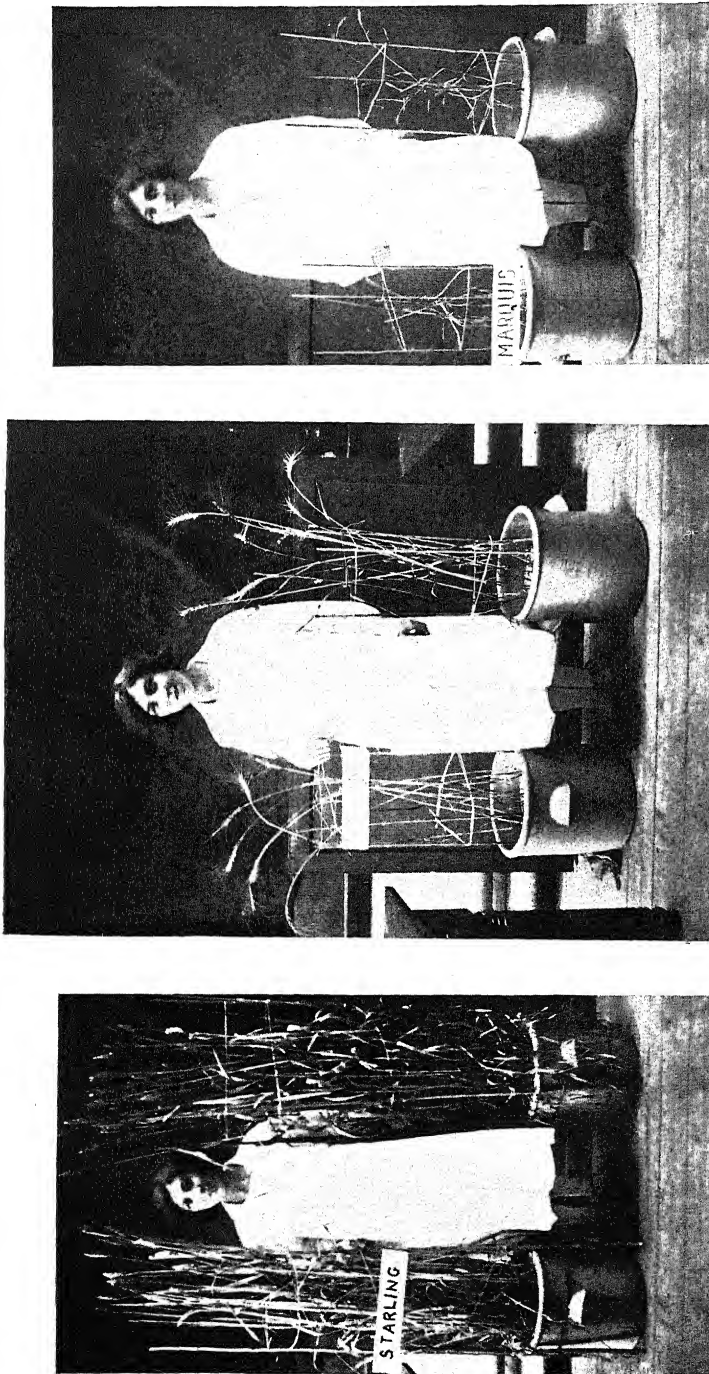
In many cases there is a noticeable parallelism of root, stem and leaf curves, pointing to the necessity of treating the whole plant as a chemical unit, so that no part of an annual plant can with accuracy be treated as an "individual." By analogy therefore it is doubtful if the "fruit spur" can be so treated. There is also a tendency for these curves to diverge during the vegetative period and to converge again after flowering or when senescence sets in.

The second most striking similarity is the parity of the C/N ratio in the three original embryos, Starling 3.8, Nevin 3.99, and Marquis 3.5. In view of the varying composition of the resulting embryos, it would be absurd to state that all wheat embryos have approximately the same C/N ratio, yet I think it probable that under

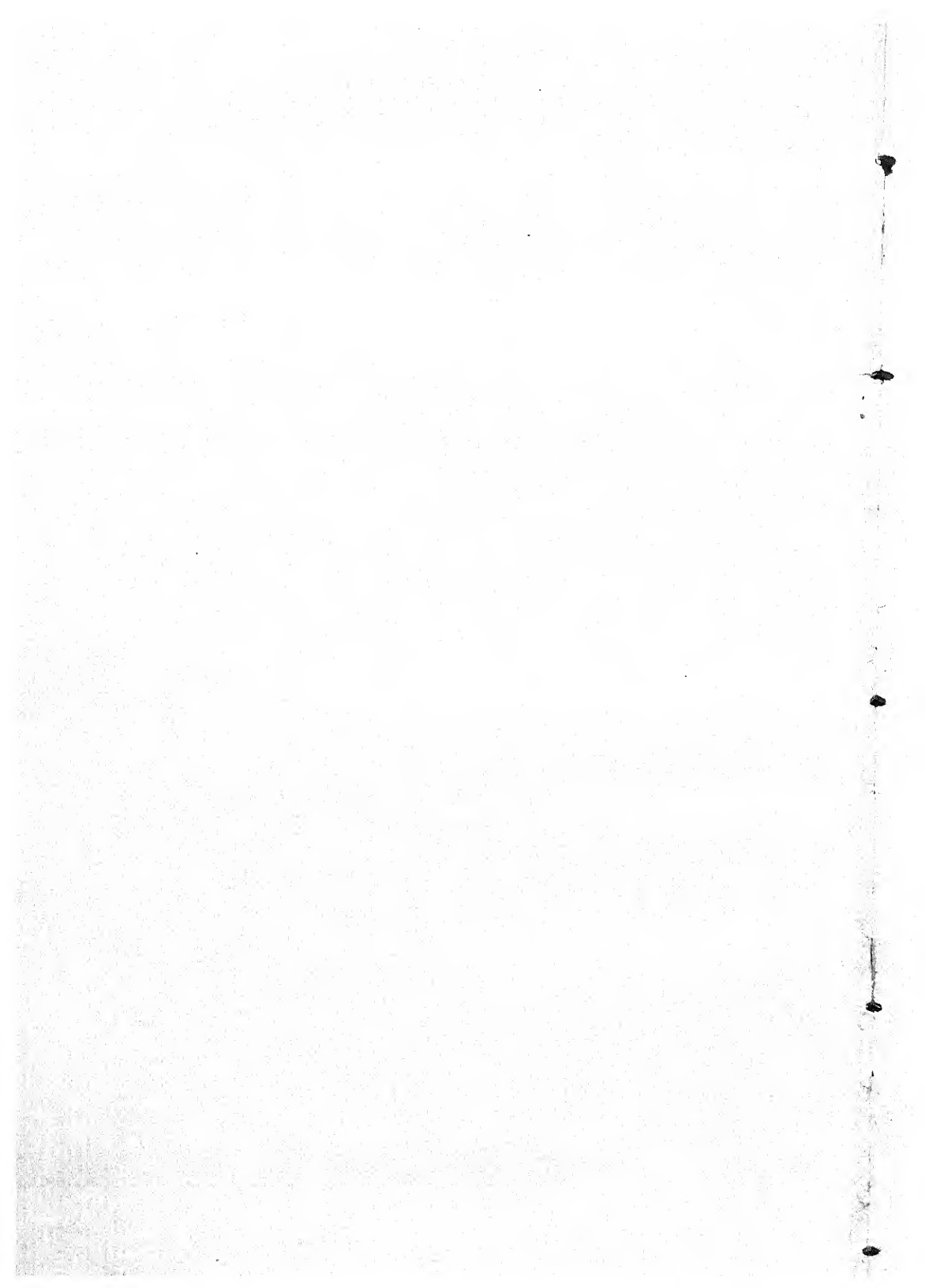
TABLE IV.

Showing the results of the analysis of different organs at various ages in "Marquis" (an American spring wheat).

Age or stage of development (see Diagrams)	Part of plant	Carbon (1) % dry wt.	Carbon (2) % dry wt.	Average % dry wt.	Nitrogen (1) % dry wt.	Nitrogen (2) % dry wt.	Nitrogen Average % dry wt.	C/N root	C/N stem	C/N leaf	C/N other parts
Seed (ungerminated)	Endosperm	33.155	33.127	33.142	2.203	2.279	2.241				
	Embryo	37.2727	37.2727	37.2727	10.48	10.57	10.525				
3 days	Embryo	36.166	37.6333	36.901	10.59	10.42	10.505				
4 days	Embryo	36.324	36.3636	36.344	10.453	10.188	10.3205				
6 days	Plumule	34.236	33.506	33.806	7.624	7.291	7.458				
	Radicle	31.518	—	31.828	4.834	4.834	4.834	6.6			
7 days	Plumule	31.909	32.358	32.133	6.1127	—	6.1127				
	Radicle	32.749	30.137	31.438	3.4185	4.178	3.798	8.2			
10 days. First leaf (transferred to culture)	Plumule	31.349	31.349	31.349	4.373	—	4.373				
	Radicle	28.247	29.175	28.711	2.965	3.224	3.0945	9.3			
14 days	Plumule	33.160	—	33.160	5.777	5.838	5.808				
	Radicle	27.227	27.227	27.227	2.723	—	2.723				
25 days. Third leaf stage	Stem	37.108	35.100	36.109	3.850	3.825	3.837				
	Radicle	33.731	30.000	31.865	2.717	2.744	2.730				
	Leaves	35.581	24.000	25.427	3.700	3.567	3.634	11.6	9.4	6.9	
45 days. Fifth leaf stage	Stem	35.616	35.030	35.323	2.255	2.255	2.255				
	Radicle	31.0909	—	31.0909	1.726	2.806	2.266				
	Fifth leaf	30.148	29.514	29.392	2.884	2.9582	2.9211	13.7	15	12.1	
66 days. Ear stage	Leaves 1-4	31.6363	31.791	31.7136	2.208	2.027	2.118				
	Stem	36.70	35.865	36.282	2.550	2.562	2.558				
	Radicle	31.133	30.142	30.908	2.279	2.279	2.279				
	Leaves	33.112	33.142	33.127	2.408	2.279	2.279				
	Ear	37.016	37.015	36.0155	2.092	2.058	2.074	13.5	14.2	14.9	Bar 17
73 days	Ovary	35.932	36.084	36.008	3.901	3.793	3.847	—	—	—	9.4
80 days	Embryo	40.619	40.564	40.591	5.79	5.626	5.708	—	—	—	7.1



Height growths of the three strains of wheat employed—"Starling," "Nevin" and "Marquis"



similar conditions of culture, the embryos would tend towards the same C/N ratio, whatever the strain or the composition of the original embryos.

The three sets of seeds were probably from plots under exactly similar cultivation. At the end of the experiment, too, the Marquis and Nevin embryos, which had taken approximately the same length of time to develop and consequently received the same amount of food, showed a similar ratio, i.e. Marquis 7.0, Nevin 7.1, whereas Starling with its longer life history received more nitrogenous food and had the lower C/N ratio of 5.7. The lowering of the nitrogen content of the embryos must have been due to starvation in this element since the carbon was the same as initially.

Under absolutely favourable conditions the C/N ratio for initial and resultant embryos would probably be the same and identical with that in any other strain grown under exactly similar conditions.

Winter wheat embryos are characterised by a higher carbon and a higher nitrogen content than those of spring sown strains. This agrees with the statement of Hedlund (27) that varieties of wheat with a higher percentage dry weight are more winter hardy than those of lower dry weight. He shows too that a higher percentage dry weight is due to high carbon content. Its most important significance is, I believe, that it compensates for the longer time of the seedling stage under winter conditions, where both carbon and nitrogen percentages are lost at such rates that the embryonic C/N ratio is maintained until the developing plumule is able to support itself and feed the plant.

Another distinctive difference is the rate of carbon accumulation in the three strains. In the winter wheat it accumulates very slowly and the plant maintaining an intermediate value of C/N is vigorously vegetative. However a much higher C/N is required to produce flowers in the winter wheat, for whereas 14-17 covers the range of conditions favouring flowering in both the spring wheats, a ratio of 31 is required by Starling.

It is obvious, too, that actual age is not the controlling factor in C/N determination, but the C/N value depends rather upon the stage of development, and the stage of development depends upon the ratio. A 4-day Marquis seedling shows the same ratio as the 7-day Starling seedling, i.e. development controlling C/N but as vegetation progresses the C/N ratio exerts its influence upon the stage of development and each strain has its own particular C/N value at which flower primordia are found.

The division of the life history into three periods, at first arbitrary, is justified by the results as a perfectly natural one, each period representing a complete metabolic cycle with a definite beginning and end point.

These results seem to explain the rejuvenescence of the plant by the sex-act. In the immediately post-sexual state, the fusion cells are as moribund as the rest of the tissues, but by some method, some inherent force of attraction, or whatever it may be, they acquire the chemical composition of a growing point, namely a low C/N ratio, due to increased carbon coupled with a considerably greater increase in nitrogen in proportion.

The work accords, with the few exceptions already pointed out, generally with the results of previous workers. It explains most of Kraus and Kraybill's<sup>(32)</sup> assumptions and the conclusions of the horticulturists, although in annuals at any rate high carbon is not to be taken alone as causative in fruit bud formation as most of them seem to think. The effects of shading and light intensity are also subject to a slight alteration in interpretation.

Shading is supposed to be effective in prolonging vegetative activities by decreasing the amount of photosynthesis, retarding carbohydrate accumulation, and hence maintaining a low C/N ratio.

But much more important than that, is the fact that shading, weak light or a short day increases the absorption of nitrates. They also encourage the translocation of the proteins from the leaves to the stem. Thus the stem and in particular its apex, as the translocation is more upwards than downwards, is kept with a low C/N ratio for a longer period and flowering is delayed.

Senescence and senescent tissues are accompanied by a high C/N ratio, chiefly through a low absolute nitrogen content, the store having presumably been used up in flower development. The value of this fact lies in the possibility of preventing senescence by controlling the nitrogen content. This of course has been done before though at the expense of flowering. But may it not be possible to apply nitrogen to annual plants, in such proportions and at such periods as would first of all allow of flower production and seed ripening and then prevent senescence or induce a rejuvenescence in the moribund vegetative tissues? These problems are under investigation and it is hoped that by careful comparison with the chemical phase changes, particularly the post-sexual phases, in perennial plants, it may become possible so to control the natural life-period as to break down the



pre-existing distinctions between the annual, the biennial and the perennial life habit.

#### SUMMARY AND CONCLUSION

A study of the C/N relations has been made during the development and senescence of three strains of wheat with varying lengths of growth period. Total elemental carbon and total elemental nitrogen were estimated by microanalytical methods. The following conclusions were obtained.

All strains if grown under exactly similar conditions tend to produce embryos with similar C/N ratios irrespective of the actual amounts of carbon and nitrogen in them; and whatever their length of growing period.

Under ideal conditions embryos are true to maternal type in the carbon and nitrogen content. Their composition is affected by nitrogen shortage.

Early stages of germination are characterised by a low C/N ratio which is kept constant even though there is a rapid percentage loss of carbon and nitrogen.

A high carbon and high nitrogen content of the embryo make for winter hardiness and compensate for the longer period of germination.

✓ A low carbon, medium nitrogen and low C/N ratio encourages vegetative growth. Vegetative activity reduces the nitrogen percentage steadily, but the carbon rises to a maximum about half-way through the life history, although the time of the maximum is liable to be affected by external conditions. Carbon percentage falls considerably before blooming. This explains the double carbon maxima which worried the American horticulturists for apple spur results, since carbon maxima in themselves have nothing to do with flower formation. The C/N ratio steadily rises throughout the vegetative period. When a sufficiently high C/N ratio is obtained, flowering occurs. Strong support is given to Kraus and Kraybill's words, "Fruitfulness is associated neither with the highest nitrates, nor the highest carbohydrates, but with a condition of balance between them."

Each cultural strain has its own distinctive C/N ratio value at which flowers are initiated but in every case it represents the maximum of the ascending C/N ratio curve.

During flowering the young ovaries pile up carbon and nitrogen reserves which pass into the embryo. The young embryo is enriched in carbon and nitrogen probably at the expense of the vegetative

organs although one may not overlook the possibility that the fertilised ovum may have the power of fixing free atmospheric nitrogen for itself. Further evidence for or against this is needed. By the proportionately greater increase of nitrogen to carbon the embryo acquires a low C/N ratio. Rejuvenescence is thus caused by the return of a detachable part of the plant to a chemically younger state, i.e. a low C/N ratio.

Conditions for the initiation of flower primordia (high C/N, low nitrogen) are the reverse of those required for fruit development (low C/N, high nitrogen).

The C/N ratio of meristem shows a gradual increase with age, reflected in the increasing initial C/N of developing organs.

Senescent tissues have a high C/N ratio; meristematic tissues a low C/N value. Hence follows a general rule for developing annual plants.

"The younger the tissues the lower the C/N ratio."

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# ILLUSTRATIONS OF CARPEL POLYMORPHISM. I

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(With 55 figures in the text.)

THE types dealt with in the following pages afford further confirmation of the theory of Carpel Polymorphism. As the families to which they belong have already been treated at some length elsewhere<sup>1</sup> a brief account of the special points of interest in each case will suffice.

## CRUCIFERAE

*Bunias Erucago* L. (Figs. 1-10). The ovary of this species has four equal sides with four strongly projecting wings at the angles, features which become accentuated as the fruit develops (Fig. 1). The flat sides stand in the orthogonal planes and thus the median suture characteristic of the typical cruciferous gynoeceium is here lacking. Further, the ovary is unique among the Cruciferae in possessing four loculi as a constant characteristic. A series of transverse sections shows that the four loculi wax and wane in succession, with the result that all four are not seen, as a rule, at any one level. Either three or two appear according as the section is taken through the middle region of the ovary, or from a higher or lower level (Figs. 2-10, in which  $l_1 l_2 l_3 l_4$  indicate the several loculi). The four sides of the ovary are veined alike, each showing in cross-section a midrib and pair of lateral veins cut transversely, while secondary veins cut obliquely or longitudinally are seen running out into the wings which represent the four sutures. The four-carpelled construction of the typical crucifer gynoeceium is thus beautifully illustrated by this species. On the old view that two carpels only are present the *Bunias* construction can only be explained on the supposition that an originally falsely bilocular ovary

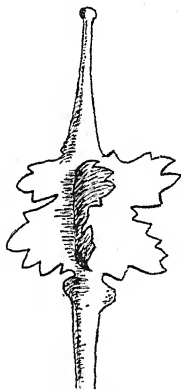
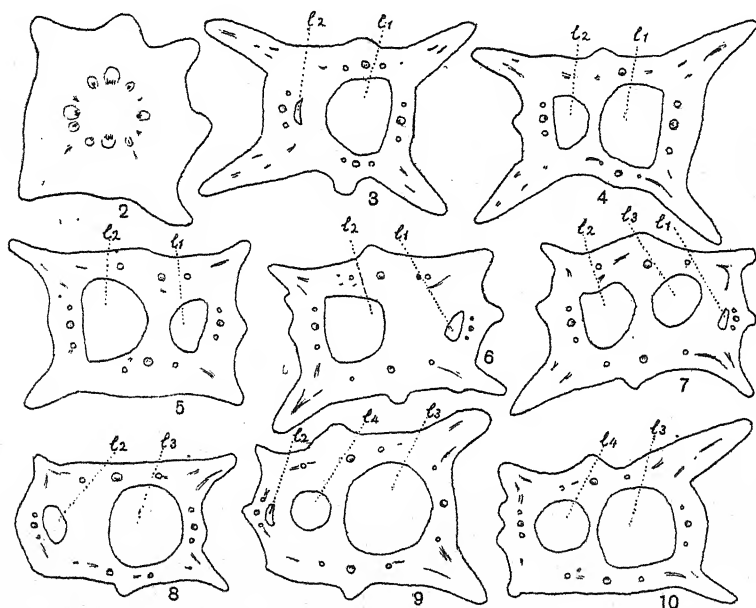


Fig. 1. *Bunias Erucago* L. Young fruit.

<sup>1</sup> *Annals of Botany*, 37, p. 457, 1923 and 39, p. 123, 1925.

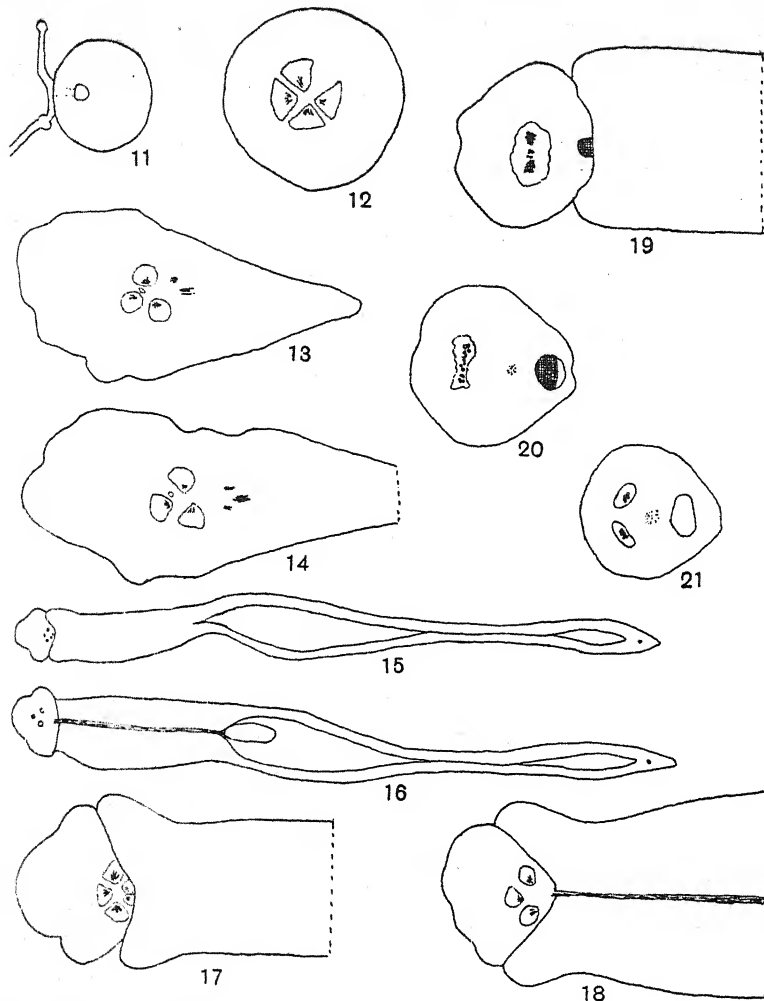
is rendered quadrilocular by the further development of secondary false partitions, and even this unconvincing explanation leaves the uniform veining of the four sides unexplained.

*Biscutella* spp. (Figs. 11-21). *Biscutella*, again, is an exceptional type, for the ovules here are not borne on the replum but spring from the edges of the valves so that the seeds are shed *with* the valves which are closed when they become detached. As on the old monomorphic view the replum is regarded as consisting of the split-off



Figs. 2-10. *Bunias Erucago* L. (continued). Transverse sections of the gynoecium taken at successive levels from below upwards showing the successive appearance and disappearance of the four loculi ( $l_1$ ,  $l_2$ ,  $l_3$ ,  $l_4$ ).

margins of two lateral valve carpels, even this normal construction presents a serious difficulty in the way of the orthodox interpretation. This difficulty becomes greater still in the case of some flowers, occasionally to be met with, in which only one valve and loculus develop instead of two, although a full-sized replum is present (Fig. 11). These exceptional flowers afford further proof, if such were needed, that the typical ovary is composed of four carpels. For, in transverse section, the non-development of the second valve is seen to be due to consolidation of one lateral carpel, as is proved by the fact that the usual four vascular cords are all present (Fig. 12)



Figs. 11-21. *Biscutella didyma* L. 11. Unusual form of gynoecium with only one valve and locus but with a normal-sized "replum." 12-21. Transverse sections of the same gynoecium taken at successive levels from below upwards. 12. Ovary base showing the usual four vascular cords. 13, 14. The lateral cord on the right is turning out horizontally preparatory to the formation of the locus. In 13 this cord has given off a marginal vein on the one side; in 14 its fellow appears as well. The left lateral cord remains in the centre, no locus or valve being formed on this side. 15, 16. Valve and locus are now seen on the right. The valve midrib appears at the far extremity of the locus while the two marginal veins remain near the centre. In 16 one of these lateral veins is seen running out horizontally to the solitary ovule. 17, 18. Portions of 15 and 16 more highly magnified. 19. A small residuum of true vascular tissue, being the continuation of the midrib of the fertile valve, has curved back over the top of the valve accompanied by a mass of mechanical tissue and appears cut across at the junction of valve and "replum." The fertile marginal veins, one of which supplied the ovule, are no longer to be seen. 20. Base of style filament showing a central area of conducting tissue. 21. Middle region of the same. The cords of the median carpels which are seen fused in 20 have separated again.

though only one member of the lateral pair turns outwards in preparation for the formation of a loculus (Figs. 13, 14). Such ovaries, in short, are characterised by having three solid and one valve carpel (Figs. 15-21) in place of two of each kind as in the normal flower.

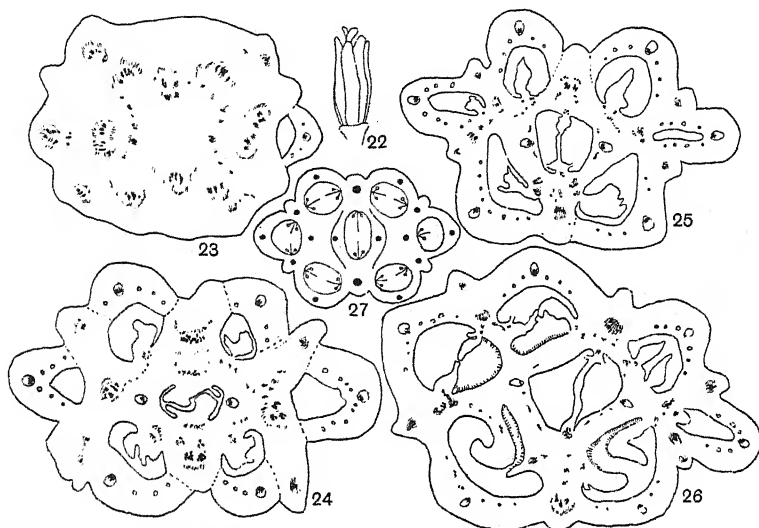
*Cheiranthus Cheiri* v. *gynantherus* (Figs. 22-27). This variety of the Wallflower is characterised by having the usual six stamens replaced by six supernumerary ovaries (Fig. 22) though further modifications of the flower sometimes accompany this primary transformation. Since the two outer stamens certainly, and probably the four inner members of the androecium also, represent each an *individual* modified leaf their replacement by a corresponding number of ovaries of *siliqua* form surrounding the normal gynoecium presents, in the abstract, a certain morphological difficulty. Examination of the simplest form of this variety showed that the replacement was accomplished through an increase in the number of floral whorls. In place of the vascular cords normally destined for the two lateral and four diagonal stamens the vascular system of the accessory gynoeical structures showed that the following carpel members were present:

G (accessory) = two lateral, valve, sterile + four diagonal, solid,  
fertile  
+ two lateral, solid, fertile + four diagonal, valve,  
sterile

in addition to the whorl of four carpels (two sterile and two fertile) of the gynoecium proper. Thus a duplication of the two whorls ordinarily constituting the androecium provides the twelve carpellary members required to produce the six supernumerary half siliquas each consisting of one valve and one solid carpel. Several interesting adjustments result from this configuration. The four diagonal *valve* carpels of the fourth whorl arising originally on the same radii as the four diagonal *solid* carpels of the second whorl (Fig. 23), as they extend to enclose the loculi, become pushed to one side where alone the necessary space is available. Hence solid and valve members originally situated on the same diagonals eventually come to stand side by side (Fig. 24). This shifting of position whereby valve carpels of an inner whorl come to extend further out from the centre than consolidated members of an outer whorl (Fig. 25) admirably illustrates how consolidation of an ante-sepalous carpel whorl in isomerous types comes to be associated with obdiplostemony.



All the solid carpels in a *gynantherus* flower may be fertile and each may form a half replum in, and send a placental bundle to, any or all of the three loculi with which it is in contact (Figs. 25, 26). As a consequence the accessory siliquas may show a single half



Figs. 22-27. *Cheiranthus Cheiri* v. *gynantherus*. 22. The gynoecium surrounded by the accessory gynoecial structures replacing the androecium. 23-26. Transverse sections of the same from successively higher levels. For the sake of simplicity the ovules are omitted. 23. From the extreme base. The xylem is becoming concentrated to form 16 carpel members disposed in five whorls as follows, 2 (*l*) + 4 (*d*) + 2 (*l*) + 4 (*d*) + 4 (*o*). Only the right member of the outermost lateral pair has, as yet, assumed valve form. 24. Both members of the outermost whorl have now formed valves. The four-valve carpels of the fourth (diagonal) whorl now stand side by side with the four solid carpels of the second (diagonal) whorl. "Replum" structures of various form are seen in the loculi of the accessory gynoecial structures as well as in the gynoecium proper. 25. The fourth whorl of carpels now extends further out from the centre than those of the second whorl. 26. The accessory carpels are separating from the gynoecium proper the surface thus exposed being covered with hairs. 27. Diagrammatic representation of the same at a level at which the gynoecium proper is partly defined but is still continuous with the accessory carpels. *d*, diagonal, *l*, lateral, *o*, orthogonal.

partition, or a normal complete septum, or two half partitions side by side. Towards the apex the supernumerary siliquas become partially separated from the central ovary by the thinning out and ultimate splitting of the ventral wall through the midrib, thereby leaving a space between the central siliqua and themselves (Fig. 26). These relations are shown in more diagrammatic form in Fig. 27.

## PAPAVERACEAE

For our present purpose this family may conveniently be subdivided into four groups as follows:

(1) Genera with a cruciferous type of gynoecium, i.e. with two valve and two solid carpels, and commissural stigmas, as e.g. *Chelidonium*, *Glaucium*, *Bocconia*, *Macleaya*, *Sanguinaria* and the Fumariaceae.

(2) Genera with more than two (usually 4-10) alternating valve and solid carpels also with commissural stigmas, as e.g. *Papaver*, *Meconopsis*, *Roemeria*, *Argemone*, *Romneya*.

(3) *Eschscholzia* and its allies *Dendromecon* and *Hunnemannia*.

(4) *Platystemon* and perhaps *Meconella*<sup>1</sup> (formerly included in *Platystigma* Benth.).

With regard to group (1) there is nothing of importance to add to the account already given elsewhere<sup>2</sup>.

In group (2) *Romneya Coulteri* Harv., with its giant flowers, shows an exceptional amount of residual vascular tissue after the stamen traces have left the central cylinder<sup>3</sup>. Here, alone among the types cited in this group does the residual vascular cylinder show alternating with the unusually massive cords of the solid carpels other small bundles which presently turn outwards to furnish the midribs of the valve carpels (Fig. 29). The stigmas here, as in the other genera included in groups (1) and (2) and as we should expect, are commissural although Eichler<sup>4</sup> and Fedde<sup>5</sup> erroneously describe them in *Romneya* as alternating with the placentae. But in *Papaver* (Figs. 30-33), *Meconopsis* (Figs. 34, 35), and *Argemone* the valve carpal midribs have gone much further, so to speak, downhill, for at the corresponding level in these ovaries they are lacking altogether. In this instance we gain something by following the course of the carpal trace in the reverse direction, i.e. from above downwards, for at a higher level a median bundle is clearly visible. As these bundles are traced downwards they are found to end blindly in the parenchyma outside the central vascular ring which they never reach, the phloem ceasing first, leaving a few unaccompanied

<sup>1</sup> I have so far been unable to obtain material of this genus for investigation.

<sup>2</sup> *Loc. cit.*

<sup>3</sup> So considerable is the amount of vascular tissue in this genus that a good deal is discarded and becomes reformed into a small secondary axial ring which persists for some time in the centre of the gynoecium.

<sup>4</sup> *Blüthendiagramme*, 2, p. 192.

<sup>5</sup> *Pflanzenreich*, 4, 104, p. 38.

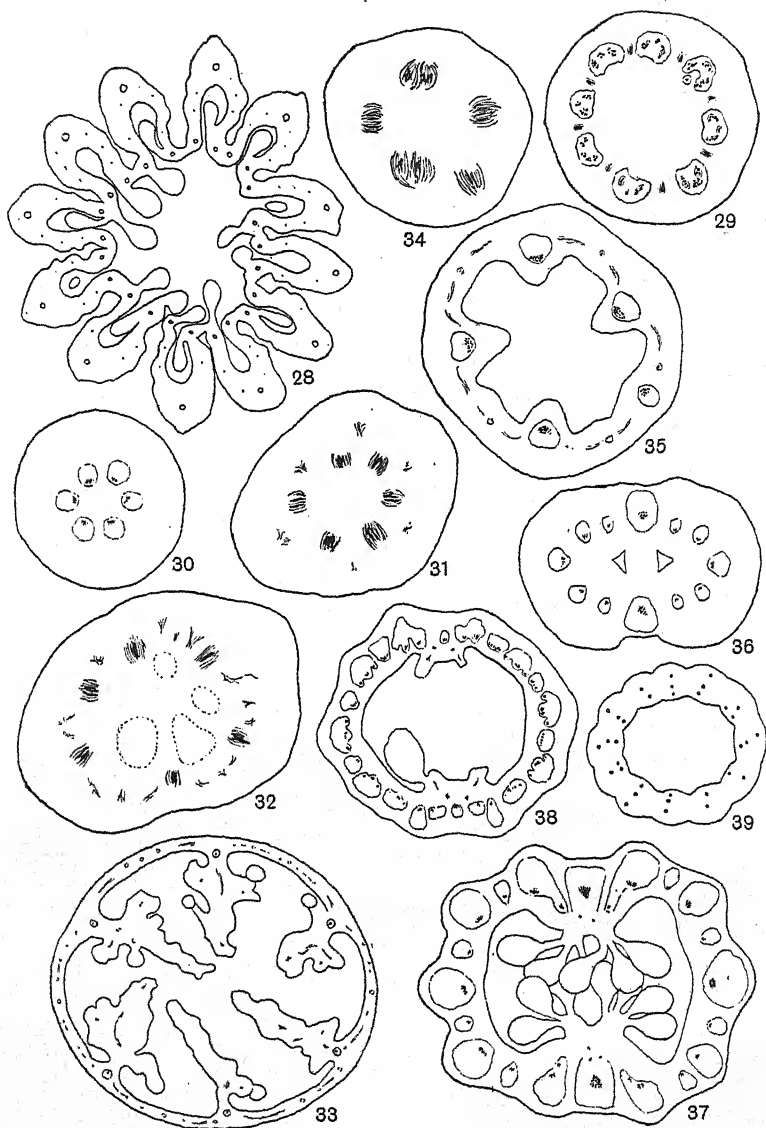
tracheides which then, too, shortly come to an end (Figs. 31, 32). On the old monomorphic view, that the solid carpel cords represent merely the coherent marginal veins of two valve carpels, we should be constrained to believe it possible that such secondary veins can continue down for some distance as independent massive cords forming a central cylinder in cases where the midrib itself only comes into being, and then perhaps as a solitary tracheide, high above this level and free in the parenchyma outside the vascular ring!

*Eschscholzia californica* Cham. (Figs. 36-39). The chief features bearing on the interpretation of the gynoeceium in this most interesting type have been previously described at some length, nevertheless, certain additional facts corroborating the view that instead of  $G\ 2$  we have  $G\ 12 + 8$  (the full ground plan being  $G\ 12 + 12$ )<sup>1</sup> call for brief mention. As in the preceding group of genera only the vascular cords of the twelve solid carpels (i.e. the median pair constituting the replum together with the five forming the ribs in each of the two lateral compound valves) are to be found in sections taken through the ovary base (Fig. 36). There is no sign at this level of the valve carpel midribs which, at a slightly higher point, are seen to alternate with the solid carpels, standing a little further in to the centre (Fig. 37). This position of the valve carpel midribs indicates that they constitute the *inner* of the two whorls. This is confirmed by the fact that the twelve members of the innermost ring of stamens, which form bulges on the inner free margin of the androeceium "collar" (Fig. 39), lie on the radius of the furrows in the outer surface of the ovary corresponding to the valve carpels, and between the projecting ridges formed by the solid carpels. What more natural, one is fain to ask, than that the 12-merous whorls in the androeceium should be followed by 12-merous whorls in the gynoeceium? Further, it is to be noted that in the upper part of the gynoeceium the midrib character of these rib cords is confirmed by the formation in the case of the stronger members of true marginal veins, a lateral xylem strand being given off on each side of the main bundle (Fig. 38).

Both *Dendromecon rigidum* Benth. and *Hunnemannia fumariaefolia*<sup>2</sup> Sweet show the same plan of ovary construction as *Eschscholzia*.

<sup>1</sup> The valve carpel on either side of the replum, back and front, is missing.

<sup>2</sup> In *Hunnemannia* the stamens are arranged in 24 radial groups. In the gynoeceium an irregular ring of tracheides, unaccompanied by phloem, appears round the central conducting tissue just below the level of the 4-lobed stigma. If additional inner carpels had existed in some ancestral form, and had later undergone degeneration in the way in which the valve carpels in *Papaver* types are degenerating to-day, such last traces of xylem, alone, and ending blindly below in ground tissue, are what we might well expect.



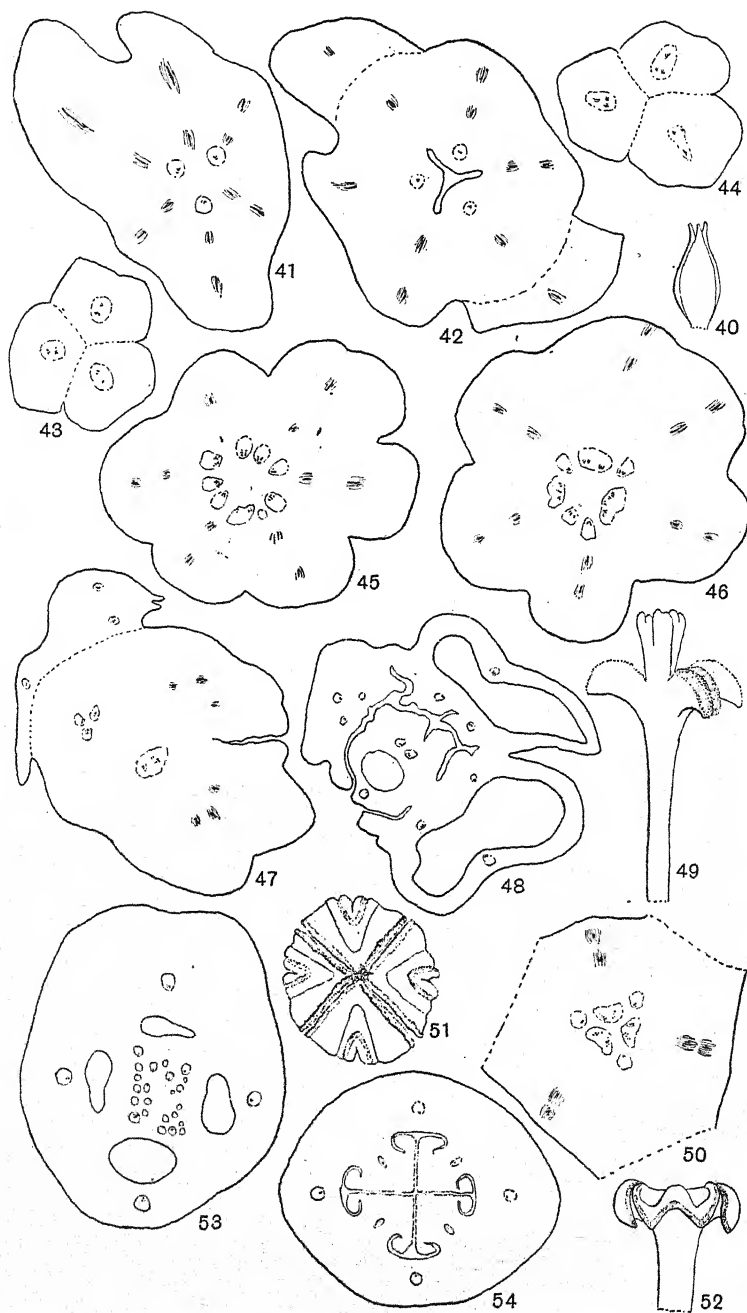
Figs. 28-39. All from transverse sections of the gynoecium in Papaveraceae except 39. 28. *Platystemon californicus* Benth. 29. *Romneya Coulteri* Harv. Ovary base; the small bundles of the valve carpels alternate with the massive cords of the solid carpels. 30-33. *Papaver Rhoeas* L., from a flower with six stigma rays. 30. Ovary base with only solid carpel cords. 31. The valve carpel bundles are seen outside the ring of the solid

On the old interpretation that in all three genera  $G = 2$  the above significant facts are left out of account and their bearing ignored. On the polymorphic view, on the other hand, it becomes clear that the gynoeceium here, as in the genera classed in groups (1) and (2), is constructed of alternate whorls of fertile, solid, and sterile, valve, carpels. That what are recognised as valve carpels in these latter genera have their counterpart in the narrow valves between the ribs of the ovary in *Eschscholzia* and its allies. Further, we may conclude from this correspondence that the valve carpels in all these types constitute a second, inner whorl which is now generally but feebly developed and is tending to lose its vascular tissue.

In *Platystemon* (Fig. 28) we have another type of great interest from the present standpoint, for in this species we appear to have an *approach* to that condition which, upon the polymorphic view, we must now consider to be of rare occurrence, viz. that of a syncarpous ovary showing parietal placentation in the original sense of the term. In the case of the *completely* syncarpous gynoeceium it now seems doubtful whether this mode of placentation, which, on the monomorphic view, is envisaged as *characteristic* of the unilocular polymerous ovary, ever actually occurs. But the degree of syncarpy in *Platystemon* is of the slightest. Partly, possibly, on this account, but more especially in consequence of the presence of an active cambial ring at the level of differentiation of the valve midribs, it is not easy to determine whether each fertile vascular strand really represents the marginal vein of a valve carpel, or corresponds to the half midrib of a solid carpel. What is, however, beyond doubt is the fact that the stigmas are here superposed upon the valve midribs.

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carpel cords and alternating with them. 32. The valve carpel bundles have formed secondary veins. The position of four of the six loculi is now indicated. 33. From the ovule-bearing region. The ovary is now unilocular. 34, 35. *Meconopsis cambrica* Vig., from a flower with five stigma rays. 34. Ovary base showing only the solid carpel cords. 35. The valve carpel midribs with their lateral veins have made their appearance on the alternate radii. The lower solid carpel on the right is less strongly developed than the others. It produced a normal stigma ray but bore no ovules. 36-39. *Eschscholzia californica* Cham. 36. Ovary base showing the twelve solid carpel cords of which the two median alone are fertile. 37. The valve carpel midribs have made their appearance on the alternate radii. It may be that the xylem branch given off by each of the four solid carpels adjacent to the median pair is the trace for the four suppressed valve carpels. If so these carpels might almost be said to come into existence at the higher level represented in 38. 38. The sterile, solid carpel cords have each given off a pair of lateral xylem strands after the manner of valve carpels about to form marginal veins. 39. Transverse section of the staminal "collar" showing twelve radial groups of vascular bundles.



Figs. 40-54.

It may well be that here this disposition of the stigmas—unique in this family (unless it be found also in *Meconella*<sup>1</sup>)—goes hand in hand with an equally unique type of ovary structure (*Meconella*, again, possibly excepted), in which *the carpels are all of the valve type*. This is the interpretation which I suggested in the earlier accounts and which the balance of evidence seems to me to confirm.

It should be stated that Eichler represents the ovules in *Platystemon* as arranged in *several* rows on each placenta<sup>2</sup>. Were this really the case it would undoubtedly constitute a serious difficulty in the way of regarding the ovary as composed altogether of valve members, since so far as appears, not more than one row of ovules is ever borne on each margin of a valve carpel. Payer, however, states definitely that there are only *two* rows of ovules on each placental ridge<sup>3</sup> and this proved to be the case also in the flowers which I examined. One comes to the conclusion, however, that the

<sup>1</sup> See footnote 1, p. 52.

<sup>2</sup> *Loc. cit.* p. 189, Fig. 79 B, a figure unfortunately reproduced in many later works.

<sup>3</sup> *Organogénie*, 1, p. 221 and 2, Pl. XLVI, fig. 24, 1857.

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Figs. 40–54. From the gynoecium in Liliaceae. 40–43. *Tofieldia calyculata* Wahlenb. 40. The gynoecium. 41–44. Transverse sections of the flower at successively higher levels. 41. Flower base. The vascular bundles for the six members of the perianth and the androecium are seen cut longitudinally. The residual vascular tissue for the gynoecium consists of three bundles on the radii of the sepals. 42. The perianth segments are becoming free. The three ovaries have separated in the centre. 43, 44. The three carpel cords have each broken up to form two lateral xylem strands which will become the fertile marginal veins. 45–48. *Tofieldia palustris* Huds. From an exceptional flower with four carpels. 45, 46. The residual vascular tissue for the gynoecium consists of a ring of bundle masses not yet concentrated into the carpel cords. 47. The whole gynoecium is nearly free from the perianth tube. One of the ovaries is beginning to separate from the other three. The carpel cords show stages in the formation of the marginal veins. 48. The four ovaries are becoming separate from each other, three have already formed a loculus. 49, 50. *Aphyllanthes monspeliensis* L. 49. Upper portion of style with three outer, spreading, and three inner, erect, stigmas. 50. Transverse section of the flower base showing the vascular cords for the petals and antepetalous stamens (those of the sepals and antesepalous stamens have already passed out into these organs), and in the centre the three outer, sterile, and three inner, fertile, carpel cords. 51–54. *Aspidistra elatior* Blume. 51. Stigmatic plate seen from above showing eight stigmatic areas, four appearing as marginal invaginations and four as ridges reaching from the edge of the plate to the centre on the alternate radii. 52. Upper portion of the style with stigmatic plate seen from the side. 53, 54. Transverse sections of the ovary. 53. From the base showing the vascular cords of the four outer carpels in line with the loculi; the vascular bundles destined for the inner carpels are becoming arranged in four groups previous to consolidation into as many cords. 54. The cords of the inner carpels are now differentiated on the alternate radii.

all-valve carpel type of ovary was proving to be not entirely satisfactory, for it will be seen from Fig. 28 that the individual loculus is evidently not large enough to permit of the development within itself of both rows of ovules, with the result that almost as many become thrust into the central space common to all the carpels as remain in their original proper loculus. It may have been this unexpected displacement which led Eichler to infer that the ovules were disposed in several rows on each placenta.

#### LILIACEAE

The general plan of construction of the liliaceous gynoecium has been already described. As a rule two trimerous whorls of carpels are present ( $G\ 3 + 3$ ), the outer being usually either of the solid or valve type, rarely semi-solid (*Fritillaria Imperialis* L.), the inner, semi-solid. Exceptionally, dimerous (*Maianthemum*) and tetramerous (*Paris quadrifolia* L., *Aspidistra elatior* Blume) ovaries occur. I have further shown in later communications<sup>1</sup> that the partially apocarpous gynoecium in *Melanthium virginicum* L. (sub-section Veratreae of the section Melanthioideae) is, as in other genera, composed of two whorls, my earlier acceptance in the case of this species of the orthodox interpretation of a single whorl of three carpels, as the outcome of observations on herbarium material, having proved at once to be ill-founded when undried material was examined. It is therefore particularly interesting to find that in another sub-section (Tofieldieae) of this very section we do actually meet with the condition in which the gynoecium is reduced to a single whorl of three carpels of the valve type. This proves to be the case in the partially apocarpous genus *Tofieldia* (Fig. 40) and in *Narthecium ossifragum* Huds. which differs, however, from *Tofieldia* in being syncarpous with loculicidal dehiscence. The accompanying figures of *Tofieldia calyculata* Wahlenb. and *T. palustris* (Figs. 41-48) illustrate the difference in structure between these exceptional types and the more usual case.

Two other genera exhibit unusual features which give fresh proof of the presence of two carpel whorls in the other sections of the family, viz. *Aphyllanthes* and *Aspidistra*.

*Aphyllanthes monspeliensis* L. (Figs. 49, 50) is stated to have a tripartite stigma, each segment being furnished with a large lateral lobe at its base, but accounts give no hint as to the origin or

<sup>1</sup> *New Phytologist*, 25, p. 294, 1926, and *Annals of Botany*, 41, p. 1.



significance of these latter appendages. In fact, however, the peculiar stigma form in this species offers a most striking piece of evidence in proof of the presence of six carpel members. The outer three carpels terminate in the three spreading stigmatic lobes recognised as such. Constituting an inner whorl and alternating with these structures are three smaller erect lobes which belong to the three inner carpels. All six stigmas are functional, for pollen grains were

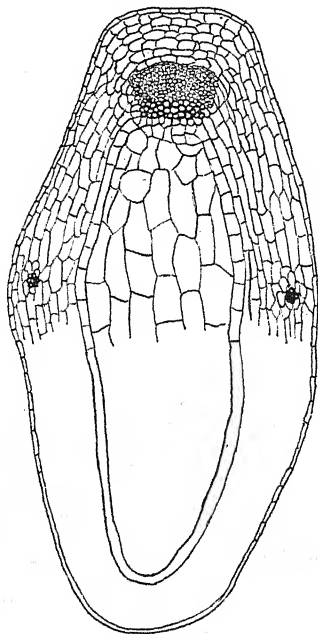


Fig. 55. *Oryza sativa* L. Transverse section of the middle region of a young fruit. The vascular bundles right and left belong to the two posterolateral carpels of the outer whorl and are prolonged up into the styles; the median massive cord belongs to the posterior fertile member of an inner whorl. The bundles of the anterior median and two antero-lateral carpels are wanting.

found to have germinated on the erect as well as on the spreading lobes in the specimens which I examined. This is particularly interesting in view of the fact that the smaller lobes receive no vascular tissue, only the outer carpel midribs which supply the larger lobes entering the long style. Also in view of the further fact that in a like case among the Eriocaulaceae (*Paepalanthus*) where six style shanks with forked stigmas are present three only are stated to be functional.

In *Aspidistra* (Figs. 51-54) the stigma is again of unusual form and is equally significant of the two-whorled plan of construction of the ovary. The short thick style terminates in a large stigmatic plate which closes the throat of the flower. In a tetramerous flower this plate shows eight stigmatic areas of which four are in the form of V-shaped invaginations of the margin on the radii of the loculi and are formed by the outer sterile carpels. Alternating with these are four other papillose areas in the form of four radial ridges on the top of the plate, reaching to its centre. These lie over the septa and belong to the four inner fertile members.

#### GRAMINEAE

In various genera of the Gramineae, which are normally two-styled, three styles are not uncommon in some of the early flowers (e.g. *Spartina*). Or where the styles are connate for a longer or shorter distance from the base three separate vascular strands sometimes enter the style filament in early flowers, instead of the normal two (e.g. *Zea*). In such genera we have evidence of the occasional development of the third member of the outer carpellary whorl which usually is suppressed. The normal two-styled flowers of these types show only one bundle on either side of the ovary in addition to the massive posterior vascular cord of the surviving fertile member of the inner whorl, as may be seen in *Spartina*, *Zea*, *Coix*, *Oryza* (Fig. 55), *Setaria*, *Panicum* among others.

The drawings here reproduced were made by Miss D. F. M. Pertz to whom I here tender my very grateful thanks.

# SOME OBSERVATIONS ON FREE-GROWING FUCOIDS

By GLADYS L. NAYLOR, B.Sc.

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(With 4 figures in the text.)

THE coastal conditions obtaining in certain regions of the west of Scotland are peculiarly suitable for the occurrence of loose-lying seaweeds, owing to the number of landlocked bays and to the shelter afforded by outlying islands.

During August 1927 several free-growing seaweeds were observed round Arisaig on the coast of Inverness-shire. The most interesting of these is a free-growing form of the common *Fucus serratus*. This would appear to be a form hitherto undescribed, and no specimens resembling it are to be found in either the Kew Herbarium or the British Museum. A short account of it may therefore prove of interest. This, including a description of the locality, will therefore be given first, while some points of interest in connection with the other loose-lying seaweeds of the district will be dealt with in the later part of the paper. Using Baker and Bohling's<sup>(1)</sup> classification, the new form will be termed *F. serratus megecad limicola*.

## HABITAT

*F. serratus megecad limicola* was observed in two localities near Arisaig. It was first noticed within the sheltered Arisaig loch, but was later found growing far more luxuriantly in a sheltered coastal cove about five miles distant (Rhue Pier).

Arisaig loch is comparatively shallow, with a long foreshore composed of muddy sand and shingle. The five common species of Fucaceae characteristic of the littoral region of sheltered coasts are abundant, both on the numerous rocky promontories and on the foreshore itself.

These are, in descending order of vertical distribution:

*Pelvetia canaliculata* L.

*Fucus platycarpus* Thur.

*Ascophyllum nodosum* L.; also var. *Mackaii* Turner.

*Fucus vesiculosus* L.

*F. serratus* L.

Owing to the sheltered and enclosed nature of the loch, there are no waves to scour the foreshore and *F. vesiculosus* covers a wide zone of muddy shingle, growing attached to quite small stones. *F. serratus* only occurs near the lower level of the neap tides and then chiefly on the larger rocks. The free *F. serratus* is found lying loosely on the muddy sand among the attached plants of *F. vesiculosus* and *F. serratus*. Its zone of occurrence extends to a slightly higher level than that of the attached form.

The second locality, known as Rhue Pier, is outside the loch, but here also the water rises and falls with the tides without any appreciable wave action, at any rate in the summer, owing to the protection afforded by the numerous islands. The cove to be considered lies between rocky headlands, the centre of the opening being blocked by a large group of rocks which are surrounded at high tide. The landward end of the cove is therefore very sheltered. The upper part of the foreshore slopes steeply, and is composed of large stones, varying from 6 in. (15 cm.) to about 3 ft. (c. 1 m.) in diameter. Below this the floor of the cove is nearly level, and composed of muddy sand. The lower edge of the rocky foreshore is above the level of the fixed *F. serratus* zone, and this form only occurs on the seaward end of the headlands and island. The free form, however, is found around the head of the cove in a zone overlapping the lower part of the rocky, and the upper part of the sandy, foreshore. The vertical range of this zone is about 3 ft. 9 in. (1.14 m.), the upper limit being 6 ft. 3 in. (1.90 m.) and the lower limit 10 ft. (3.0 m.) below the upper level of the *Pelvetia canaliculata* zone. This was used as a standard because it was not possible to obtain data as to the spring tide levels. In the upper part of its area, the free *Fucus* is found lying underneath plants of *F. vesiculosus* and *Ascophyllum nodosum* which grow densely on all the stones. Below the stony area megecad *limicola* occurs abundantly, either as a pure formation, loose-lying or partially embedded in the muddy sand; or in conjunction with *A. nodosum* var. *Mackaii* forming extensive mats many square yards in area.

It is noteworthy that in both localities the upper limit of the free-lying form is above that of the fixed form. In the Rhue Pier area the two zones are entirely separate, but as this is the more exposed locality the action of the sea keeps the free form washed up on the beach.

#### HABIT

The characteristics of *F. serratus* megecad *limicola* agree with those which are typical of *limicola* forms in general:

- (1) Vegetative reproduction,
- (2) Dwarf habit,
- (3) Absence of attachment disc,

but in addition there is curling of the thallus which is usually associated with salt-marsh forms rather than loose-lying ones.

The free plants are considerably smaller than the fixed ones, having an average length of 25 cm. as compared with the 50 cm. of the latter. The width and thickness of the thallus are also less. In this locality *F. serratus* has a rather wide thallus, averaging 2 cm. across, while the free form averages 1.3 cm.

In texture the thallus is firmer and less mucilaginous than the parent form. This is probably associated with the greater exposure to which the free form is subjected. The thallus is much branched and curled which gives the plant a characteristic appearance, very different from the thallus of the fixed form which tends to lie all in one plane (Figs. 1 and 2).

The cryptostomata are rather fewer and smaller, but are arranged, as in the parent form, in two rows on either side of the midrib.

In colour the younger parts of the thallus are a bright yellow-brown, but the older parts are much darker.

#### ORIGIN AND REPRODUCTION

There is little doubt that the form has arisen directly by vegetative growth from broken pieces of the normal form brought up by the tide. Several such pieces were observed along the shore, although actual intermediate forms were not found. In Arisaig loch, one plant was found attached to a stone embedded below the surface of the mud. This plant showed all the characteristics of the free form, except for its attachment disc, thus affording additional evidence that the change in form is due to environmental conditions.

No receptacles were found on any of the free-growing individuals, whereas the fixed plants were fruiting well. As observations were only made during August it is possible that receptacles appear at some other time of the year. It is probable, however, that the form is entirely sterile, reproducing itself in a purely vegetative manner. It would appear that as the plants grow and branch, the lower parts

rot away, causing the thallus to separate into many pieces, each of which continues life as an individual plant.

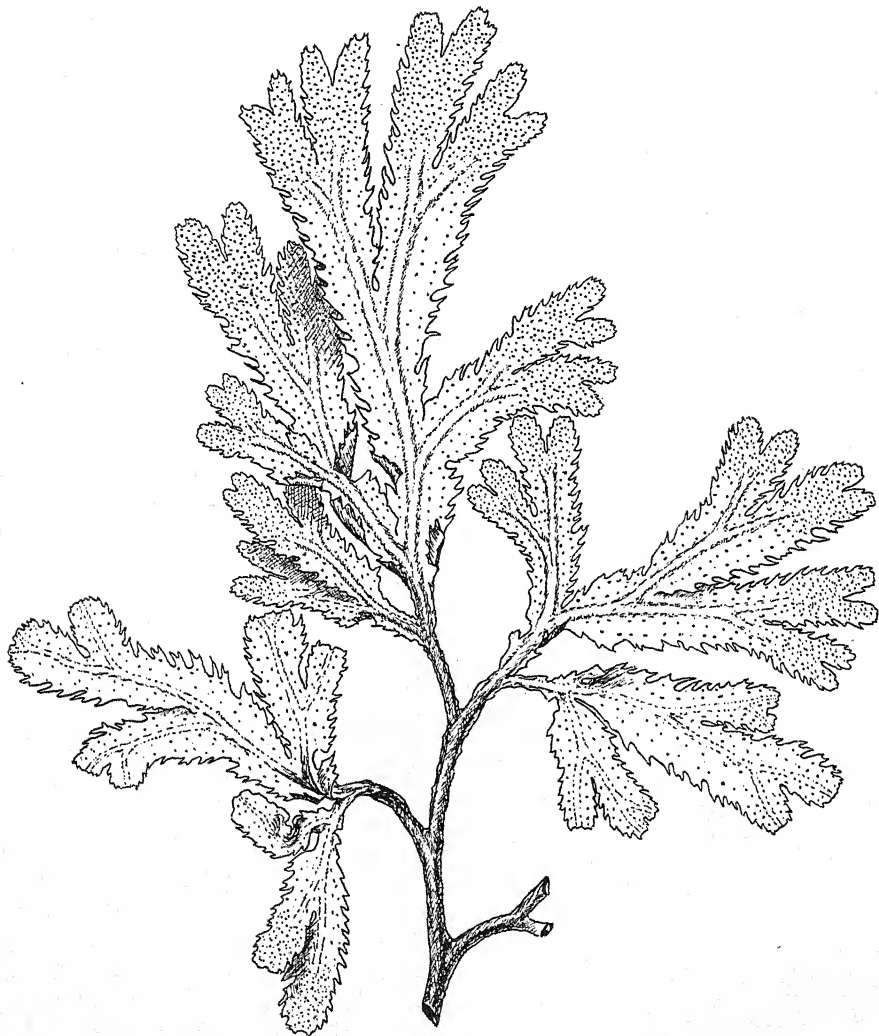


Fig. 1. *Fucus serratus*.  $\frac{1}{2}$  nat. size. Rhue Pier, Arisaig, Inverness-shire.

The occurrence of this free form of *F. serratus* is remarkable when one considers how intolerant of desiccation is the parent form. Experiments by S. M. Baker (2) showed that young plants of *F. serratus*

could not survive a period of exposure of six hours in every twelve, yet the loose-lying plants in the Rhue Pier locality are exposed regularly for nearly this length of time, and are far more prolific in growth than those within the loch where the exposure is considerably shorter.



Fig. 2. *Fucus serratus megecad limicola*.  $\frac{1}{2}$  nat. size.  
Rhue Pier, Arisaig, Inverness-shire.

#### OTHER LOOSE-LYING SEaweEDS OF THE ARISAIG DISTRICT

In addition to *F. serratus megecad limicola*, and sometimes closely associated with it, there are two other loose-lying seaweeds, *Ascophyllum nodosum* var. *Mackaii*, and a free form of *Pelvetia canaliculata*.

The latter resembles the salt marsh form *Pelvetia canaliculata* var. *libera* which occurs abundantly at Blakeney Point, but which has not been recorded outside Norfolk. In the Arisaig area there are no salt marshes and the conditions of life on the foreshore do not appear to favour the growth of loose-lying *Pelvetia*, since only isolated plants were found. Within the loch these occur at a higher level than *F. serratus megecad limicola*, but at Rhue Pier the few free *Pelvetia* plants observed lay in the upper part of the free *Fucus* zone.

*Ascophyllum nodosum* var. *Mackaii* is by far the most abundant of the loose-lying forms of the area, its characteristic appearance making it also the most conspicuous. The globular tufts in which it grows vary in size from a few inches to a foot and a half or more

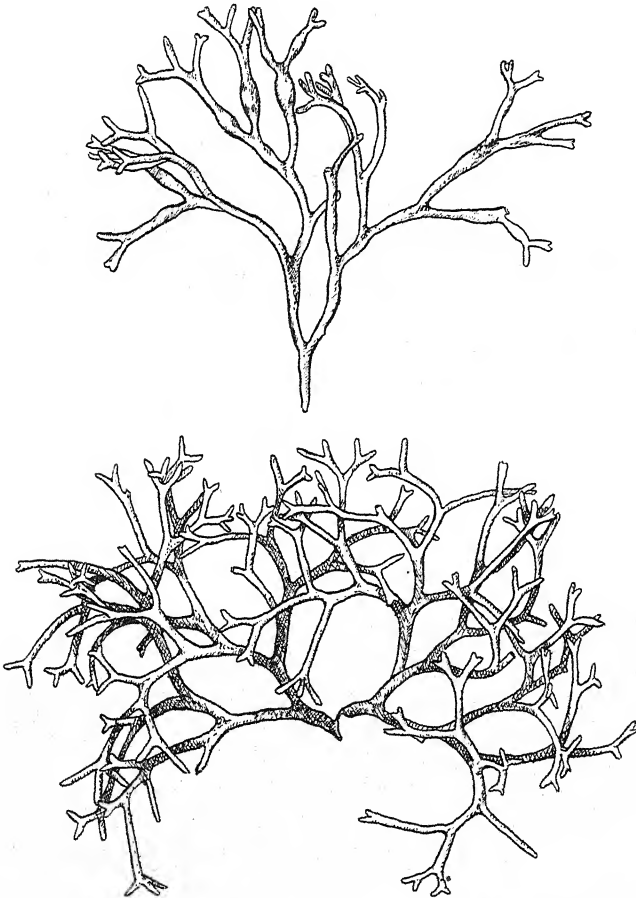


Fig. 3. *Ascophyllum nodosum* var. *Mackaii*.  $\frac{1}{2}$  nat. size.  
Arisaig, Inverness-shire.

in diameter. Air vesicles are frequently present, occurring singly just above or below the dichotomies. The vesicles are considerably smaller than those of the fixed form, the largest found being 1 cm. long by .7 cm. wide. All the plants do not produce air vesicles, and many quite large specimens were found which had none at all. It



does not appear therefore that the production of vesicles depends upon the age of the plant since their absence is not confined to the smaller specimens.

Although it has been understood for many years that *A. nodosum* var. *Mackaii* is only a form of the fixed *A. nodosum*, actual intermediate forms have not been recorded. During August, however, a great number of specimens which showed intermediate stages between the two forms were observed on the foreshore of Arisaig loch. Broken pieces of *A. nodosum* which showed vigorous vegetative growth, were found lying among the plants of *A. nodosum* var. *Mackaii*. In some cases, the new branches produced, closely resemble the smaller plants of the free form; the thallus is still rather flattened in cross-section, but frequent dichotomous branching occurs producing a tufted growth, and air vesicles when present arise singly near the dichotomies (Fig. 4 C). In most cases, however, the first branches to arise do not branch dichotomously, but give rise to numerous lateral branches which may themselves produce laterals before dichotomous branching begins (Fig. 4 A and B).

Cotton(3) suggests in his Clare Island Survey that the var. *Mackaii* only arises from the normal *A. nodosum* at certain times of the year. He worked at Clare Island during the winter and early spring, at which time the *A. nodosum* var. *Mackaii* was fruiting. It is interesting to note that while vegetative reproduction was proceeding rapidly this August, no fruiting specimens of *A. nodosum* var. *Mackaii* were found.

In both the areas considered *A. nodosum* var. *Mackaii* occurs in a zone whose vertical range is about 4 ft. 9 in. (1.44 m.). Within the loch the upper limit is 3 ft. (90 cm.) below that of the *Pelvetia canaliculata* zone, but at Rhue Pier the upper limit of var. *Mackaii* is 4 ft. 6 in. (1.37 m.) below the *Pelvetia*. It is only in this second locality that *A. nodosum* var. *Mackaii* and *F. serratus megecad limicola* occur together. The zones of vertical distribution of the two forms are as follows:

*A. nodosum* var. *Mackaii*, 4 ft. 6 in. to 9 ft. 3 in. (1.37 m. to 2.81 m.).

*F. serratus megecad limicola*, 6 ft. 3 in. to 10 ft. (1.9 m. to 3 m.).

They occur together in many parts of the beach, and it is possible that the *Ascophyllum* proves beneficial to the free *Fucus* by protecting it from excessive desiccation.

In conclusion I would like to express my thanks to Mr A. D. Cotton and Miss Duke of the Herbarium, Royal Botanical Gardens,

Kew, and to Mr Tandy of the Botanical Dept. of the British Museum, for assistance in the examination of herbarium material.

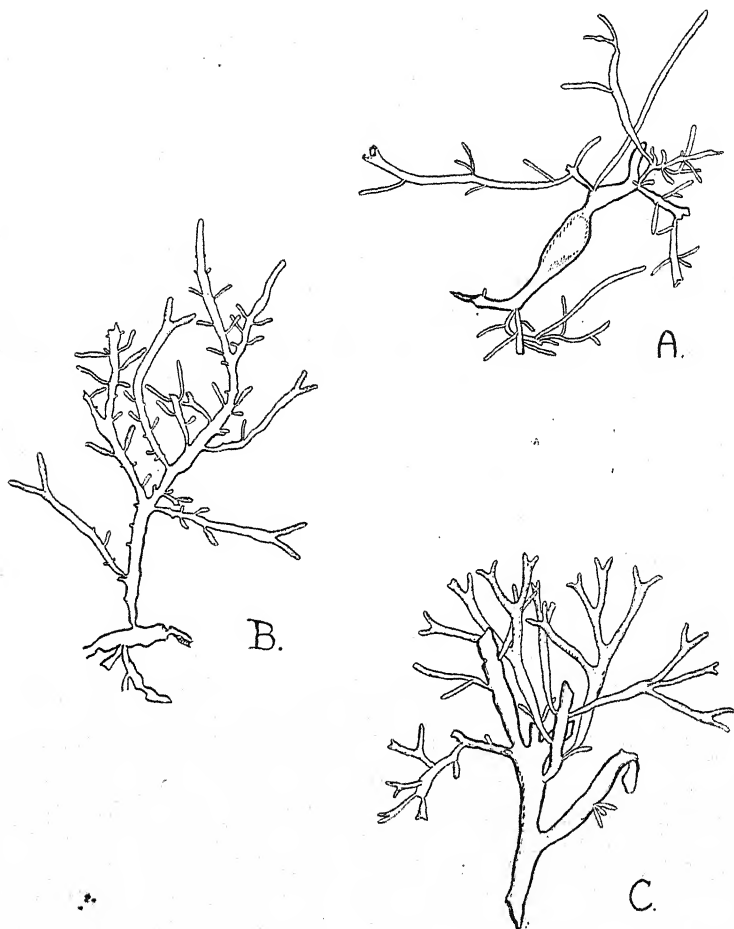


Fig. 4. Intermediate stages in the production of *Ascophyllum nodosum* var. *Mackaii* by vegetative growth from broken pieces of *A. nodosum*.  $\frac{1}{2}$  nat. size. Rhue Pier and Arisaig loch, Inverness-shire.

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DISEASE RESISTANCE IN PLANTS<sup>1</sup>

By F. T. BROOKS.

University Lecturer in Botany, Cambridge.

INDIVIDUAL human beings differ markedly in their susceptibility to particular diseases. In plants, which are much simpler in organisation than the higher animals, differences in reaction to disease are characteristic of the closely related varieties of which all crop plants consist. It is rare for the individual members of a variety of plant which breeds true to type to differ appreciably in susceptibility to a specific disease in the same locality.

Every cultivator of the soil, be he farmer, fruit-grower, or amateur gardener realises the value of growing varieties of crop plants true to type, for, apart from other qualities, he knows, for example, that the different varieties of potatoes, apples or roses differ enormously in their liability to disease.

With potatoes, for instance, the variety "President" is resistant to Blight while the variety "Up-to-date" is very susceptible; the variety "Great Scot" is immune from Wart disease while "King Edward" is badly attacked.

With apples, the variety "Cox's Orange Pippin" is very liable to Canker, while "Bramley's Seedling" is quite resistant to this disease.

With roses, "Dorothy Perkins" and "Crimson Rambler" are extremely susceptible to Mildew, while such a variety as "Gloire de Dijon" is practically never affected.

Certain kinds of cultivated plants are grown at periods of the year when they may completely escape attack from disease. Such varieties are not immune or even resistant to certain diseases, but they remain healthy because the germs of these diseases are not available in the air or soil to cause infection.

For example, early varieties of potatoes are only very rarely attacked by Blight because they are dug before the spores of the Blight fungus are present in the air. Such varieties are in fact quite susceptible to Blight.

<sup>1</sup> Lecture given at the meeting of the British Association for the Advancement of Science, Section K, Leeds, September, 1927.

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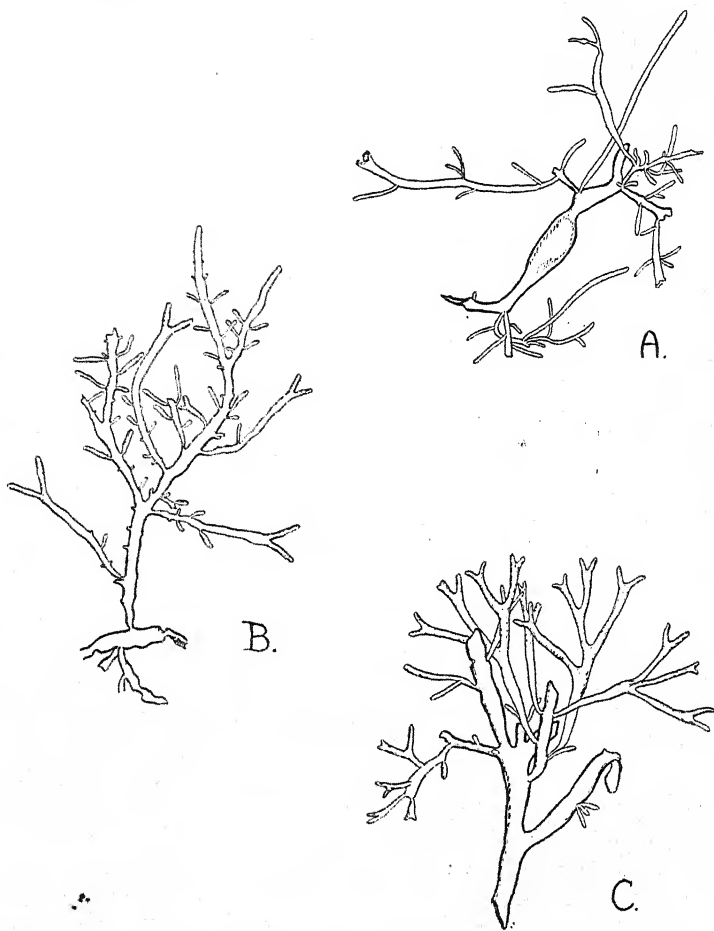


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In this country wheat is not often seriously affected by Black Rust, for, even when attacked, the fungus appears so late on the plants that little damage is done. Where, as in Canada, this fungus attacks wheat at an earlier stage in the growth of the plant, great damage is often done. If perchance Black Rust again threatened to seriously affect wheat in this country, attempts would be made by plant-breeders to introduce into cultivation varieties which would mature earlier than those now in use.

An important difference between plant and human diseases lies in the fact that there is extremely little evidence of anything of the nature of acquired immunity in plants. In recovery from certain human diseases, especially from some of those of parasitic origin, the individual is often rendered immune or very resistant to another attack for a considerable time. This is because the human organism in its struggle with the diseased condition has elaborated anti-bodies, which persist and afford a measure of protection after recovery has taken place. With plants, recovery from disease generally confers no degree of resistance against a renewed attack. A plum tree which has recovered from Silver-leaf disease may be quite readily infected with this disease again immediately after recovery has taken place.

It is a matter of common knowledge that many plants, grown at a time when the germs of disease are present in abundance, remain more or less immune from attack. It is clear therefore that such plants must have some means of protection.

It is of course conceivable that structural differences between resistant and susceptible varieties account for the prevention of attack in the former class. This is in fact partly true.

A somewhat thicker cuticle over the leaf may prevent invasion by those fungi the germ tubes of which effect entry into the tissues by penetration of the cuticle. One often sees that young leaves are attacked by a certain fungus, whereas mature leaves of the same plant are not so attacked. Thus *Puccinia graminis* can only infect young barberry leaves. The reason for non-infection of mature leaves is that their cuticle is so thick that the germ tubes cannot penetrate it.

*Botrytis* spores often infect slightly cuticularised parts such as flowers, whereas they cannot usually infect healthy, fully cuticularised organs such as mature leaves unless additional saprophytic nourishment is provided.

One of the worst diseases of apples and pears is "Scab." This

affects the twigs and leaves, and causes the unsightly, black blotches so often seen on the fruit. In susceptible varieties the germ tubes of the fungus penetrate the cuticle of the young leaf or fruit, and a mycelium is quickly established below the cuticle, which grows laterally and gives rise to conidiophores that push off the outer skin and appear as a blackish covering. Certain varieties of apple and pears are very resistant to this disease, at any rate in most seasons. In these almost immune varieties the young leaves and fruits are penetrated by the germ tubes of the spores and the fungus begins to grow laterally in the cuticle(14). Development, however, is feeble, and the fungus dies without producing conidiophores, so that to the naked eye no scab is apparent on the fruit. Here it seems that in the resistant varieties the fungus is gradually starved out, or, alternatively, that some toxic substance is secreted by the host.

The amount of sclerenchyma in the tissues may be the factor determining whether the host plant is only slightly affected or severely attacked. In the United States the variety of wheat "Webster" is highly resistant to attack by many forms of *P. graminis*, and this has been shown to be correlated with a particularly profuse development of sclerenchyma in the stem(10). The abundant sclerenchyma greatly checks the progress of the rust mycelium in the tissues.

Potato varieties show considerable differences in the susceptibility of their tubers to potato Blight. *Phytophthora infestans* infects potato tubers through the lenticels or through the eyes. As regards attack through the lenticels it has been shown that tubers which remain free from blight often have lenticels which are markedly suberised(7). Owing to the impregnation of the walls of the lenticel cells with corky substances the delicate hyphae of the fungus cannot pass through them, and invasion is barred.

Apart from the differences between young and old plant organs, all the structural features just referred to can be looked upon as being the result of essential protoplasmic differences between variety and variety. In the great majority of cases no obvious structural differences between susceptible and resistant varieties can be detected, and resistance is dependent upon protoplasmic differences too subtle to be analysed by present methods. In disease-resistant varieties of this class the parasite successfully effects entry into the tissues, but, accompanying or following this, the host reacts in some way so as to prevent the parasite from proceeding further. Thus although the initiation of infection is safely accomplished, the parasite cannot proceed further on account of the reaction of the

host. A struggle is fought out between host and parasite, and in these resistant varieties of plants the parasite is defeated.

In recent years much work has been done in trying to elucidate the nature of the struggle between host and parasite in these resistant varieties. In some cases the story of this struggle is of a surprising character.

One of the first investigations of this kind was that concerning the resistance of certain varieties of wheat to Yellow Rust. Varieties of wheat resistant to this rust such as "Einkorn" and "American Club" often show innumerable yellow flecks in the leaves, which are now known to be areas where the fungus has unsuccessfully tried to establish itself. In the variety "Einkorn," which is practically immune, the spores germinate on the leaves, and their germ tubes pass successfully through the stomata. Having safely negotiated the stomata the advancing hyphae come into contact with the cells below the stomata: the attack by these hyphae is so violent or the host cells are so weak that the latter are immediately killed (8). In this way a barrier of dead cells is erected around the fungus which the latter cannot pass, for rust fungi can only continue to live when in contact with living cells of their hosts. This is an astonishing result, and it appears that immunity from attack in such a case is due to too violent an onslaught on the part of the fungus, which prevents the establishment of the common life between host and parasite necessary for the continued existence of the latter. With the variety "American Club," which is not quite so highly resistant as "Einkorn," there is a more extended struggle: occasionally the fungus establishes itself in the tissues by sending haustoria into the still living cells, with the result that the mycelium may grow sufficiently to be able to form a few spores. In varieties fully susceptible to Yellow Rust the fungus and host cells live a common life together, and although food material is withdrawn by the fungus the host cells maintain their life.

With other wheat rusts also similar phenomena have been described: many varieties of wheat resistant to Black Rust in the United States have been shown to owe their resistance to a too vigorous initial onslaught on the part of the parasite, leading to the death of the host cells in the immediate vicinity. Workers in the United States speak of such varieties as being hyper-sensitive to the parasite.

With the variety of wheat "Mindum," which is immune in the United States to a certain form of Black Rust, the fungus actually



establishes haustoria in the cells below the stomata; these cells, however, react violently to the presence of the fungus, with the result that both the haustoria and the cells are killed (4). The infecting hyphae establish other haustoria, but as the cells again react in the same way, the infecting hyphae become exhausted and the host remains immune.

On the other hand, the resistance of some wheat varieties to these rusts may be found not to be due to initial excessive violence, but to too weak an attack, leading to failure to establish haustoria in the host cells. In this way the parasite may be starved out.

In this connection it is interesting to note that when the spores of rust fungi are put upon the wrong hosts the germ tubes successfully pass through the stomata and the preliminaries to successful infection are accomplished; but with failure to establish intimate contact with the host cells the young hyphae quickly perish (5).

In other diseases the nature of the reaction of the host in resistant varieties is entirely different. The variety of plum "Victoria" is very susceptible to Silver-leaf disease, whereas the variety "Persshore" is markedly resistant. When *Stereum purpureum*, the cause of Silver-leaf disease, attempts to infect a fresh exposure of the woody tissues of a Persshore plum the sequence of events is as follows. Many of the spores on coming into contact with the exposed surface under moist conditions are sucked into the vessels, where they germinate without danger of desiccation. A mycelium is quickly formed and this grows downwards through the wood. As with successful infection of a Victoria plum tree the progress of the fungus is accompanied by the formation of large quantities of gum from the food substances in the wood, the accumulation of which causes a marked discoloration of the wood. With the Persshore variety, however, as time goes on, so much gum is formed by the reaction of the host to the parasite that around the periphery of the invaded tissues a barrier of gum is established, which is so dense that the fungus cannot penetrate it (3). The parasite cannot proceed further and is occluded. Sooner or later it dies, and successful infection is prevented. Here also, although the initiation of infection is accomplished by the fungus, full infection does not result owing to the host's reaction.

Fresh wounds in the Victoria plum are very susceptible to infection by *Stereum purpureum* throughout the year except during the months of June, July and August. During the summer this usually susceptible variety is extremely resistant to invasion. Owing

to the tree being in a different physiological state in the summer it is able to form more profuse quantities of gum than at other times of the year. If spores of the fungus alight on a fresh wound in the wood during the summer, the initiation of infection is begun, but so much gum is produced that the progress of the fungus is quickly stopped. A gum barrier has again been formed which prevents the fungus from proceeding further. It is of interest that the phenomena associated with the inability to infect a susceptible variety during a certain period of the year are of the same kind as those associated with the prevention of invasion in a resistant variety.

It is now well known that trees affected by Silver-leaf disease sometimes regain their health. In these cases also a gum barrier has been formed by the host around the invaded tissues, which prevents the fungus from proceeding further. The result is that the fungus is confined to the zone already invaded, and it is only a question of time before it dies out.

It has been shown that with parasites which invade the woody parts of plants the excessive formation of gummy substances often prevents extensive invasion. With organisms which chiefly invade parenchymatous tissues, particularly in stems and stem-like organs, the commonest type of reaction is the formation of a cork barrier just beyond the region reached by the parasite. In general, corky cells cannot be permeated by fungal hyphae, so that these cork barriers often effectively bar the way. Familiar instances of the formation of these cork barriers are afforded in the diseases known as Larch Canker and Apple Canker. The fungus of larch canker or the fungus of apple canker progresses actively in the bark during the winter, but in the spring or summer the host temporarily checks the invader by the formation of a cork barrier. In the autumn the fungus often evades the barrier and the canker is extended. Sometimes, however, the cork barriers successfully keep the fungus at bay, especially in larch trees which are growing vigorously.

With some varieties of cultivated plants the quality of resistance to certain fungus diseases is bound up with the capacity of the variety to readily form these cork barriers in response to attempted invasion.

One of the serious diseases of cultivated flax is a wilt caused by the invasion of the root system by a species of *Fusarium* from the soil. In susceptible varieties the mycelium passes from the surface of the root to the vascular tracts, but, in resistant varieties, as soon as penetration of the root cortex has begun, a corky barrier is laid

down in the immediate vicinity, which prevents the fungus from entering the vessels(11). The consequence is that varieties able to react in this way remain unaffected by the wilt disease.

As will be explained later, the resistance of certain varieties of plants to specific diseases can nearly always be broken down by exposing the plants to unfavourable environmental conditions. Salmon(9) was able to break down the resistance of barley to the wheat form of mildew by wounding the barley leaves. With Wart disease of potatoes, however, a variety of potato immune from the disease remains immune under all known conditions. Miss Glynne(6) has recently shown that a few varieties, previously thought to be immune as the result of field observations, form very small warts when inoculated with the fungus under laboratory conditions; such varieties are therefore slightly susceptible. In susceptible types the invasion of superficial cells of the eyes of the tuber is followed by extreme proliferation and division of the neighbouring cells, with the result that a large warty excrescence is formed. With the immune types the parasite also effects entry into the surface cells of the eyes; in those varieties described by Miss Glynne as producing small warts in the laboratory there is some proliferation and abnormal division of the neighbouring host cells. In truly immune varieties the presence of the fungus causes no obvious response on the part of the host, and the fungus dies in the host cell it has penetrated. The result is that no wart is formed, and from the practical standpoint the variety is "immune." It is clear that in cases of this kind immunity is bound up with a particular protoplasmic quality of the host, which prevents the response that accompanies successful invasion of a susceptible variety.

One of the most interesting examples of disease resistance is that exhibited by coloured varieties of onions to the disease known as "smudge," which is prevalent in North America. White varieties of onions are badly affected by this disease, which causes the development of black blotches on the bulbs. Onions with a coloured skin are almost completely immune from this disease, and it has been shown that this immunity is dependent upon the anthocyanin pigments or to bodies closely associated with them(12). A solution of these pigments prevents germination or causes abnormal germination of the spores, and is also highly toxic to the mycelium of the fungus. If the red skin of an onion is wounded, so that cells devoid of pigment become exposed to the fungus, the disease is established as readily as in white varieties. It is suggested that in nature small

quantities of the pigments diffuse out from the dead outer cells of the coloured varieties and inactivate the fungus in the adherent soil.)

In discussing Wart Disease of potatoes it was indicated that, generally speaking, the resistance of varieties of cultivated plants to specific diseases was modifiable within certain limits according to environmental conditions. In dealing with any kind of parasitic attack we have to take into consideration both the inherent constitution of the host with regard to resistance and the particular environmental conditions under which the plant is grown. The latter may be unfavourable to the host, preventing it from reacting to the parasite in the usual manner, thereby allowing disease to become established, or, alternatively, the environment may particularly favour the fungus in some way. The result in either case is the establishment of disease in what is usually a resistant variety. Sometimes the degradation of the variety is so marked that its inherent resistant character is almost completely masked.

Thus, if varieties of wheat normally resistant to Yellow Rust are grown in soil which is manured excessively with nitrogen, it is practically certain that they will become moderately affected. "Einkorn" wheat, which is almost immune to Black Rust as well as to Yellow Rust under ordinary conditions, may be severely affected by Black Rust in the Ganges Valley during the very hot weather of May. In both instances the inherent resistance has been profoundly modified, in one case by the chemical nature of the soil and in the other by temperature. We do not yet know precisely in what way this marked alteration in response of the host to parasitic attack is brought about.

In general there are two chief groups of factors in the environment which are capable of modifying the inherent resistance of a plant. These are soil and weather. The conditions of soil and weather are partly interdependent, for both rainfall and air temperature affect soil conditions. There are, however, certain soil factors, such as physical texture and chemical nature, which are practically independent of weather, so that it is often necessary to enquire which particular component of the whole environment is chiefly responsible for the adverse influence on the plant. Recent investigations have stressed the enormous importance of environmental influences upon the incidence of plant diseases.

It has already been stated that nitrogen in excess tends to render wheat more liable to attacks of rust. On the other hand, salts of potassium in slight excess increase resistance to rust fungi.

On heavy soils, retentive of water, varieties of apples usually free from Canker often become seriously attacked by this disease.

In connection with the soil it is now recognised that with those fruit trees like the apple and plum which are budded or grafted on stocks of a different kind, the nature of the root system of the stock exercises a profound influence on the growth of the upper part of the tree. In some cases the nature of the stock affects markedly the susceptibility of the variety to certain diseases. The variety of apple "Bramley's Seedling" is usually extremely resistant to Canker, but a short time ago I saw a number of trees of this variety, worked on an unusual stock, which were so badly cankered that some of them died. Here again we do not know in what way the stock exercised the adverse influence, though this was probably bound up with the nature of the root system.

Whereas on poor soils the larch tree is extremely liable to Canker, on soils of good quality, where the situation is otherwise suitable, Canker is almost non-existent. This is because the tree, when growing vigorously, produces cork barriers so rapidly and effectively that any considerable progress of the fungus in the bark is prevented.

Temperature is the weather factor which probably exercises the greatest influence in modifying disease-resistance in plants. Each living organism thrives best within a certain range of temperature; a little above or below this range it may continue to live with diminished vitality. Lack of vigour at unfavourable temperatures may render a normally resistant plant quite susceptible to disease. A certain parasitic fungus may have quite a different temperature for optimum growth than its host; if therefore the temperature be particularly suitable to the fungus and detrimental to the host, disease of a serious kind will probably ensue. Under reverse conditions, i.e. favourable to the host and detrimental to the fungus, there will be little or no disease.

One of the most striking investigations upon the influence of temperature on disease-resistance is that carried out recently in the United States upon the seedling blight of wheat and maize, caused by *Gibberella Saubinetii* (4). As is well known, wheat grows best at relatively low soil temperatures, whereas maize thrives best at high temperatures. The fungus which causes seedling blight of these plants grows well over a wide range of temperature. On soil infected by this fungus wheat remains unaffected if the seed germinates at a temperature of about 8° C. If, however, the wheat begins to develop at a high soil temperature (20°–28° C.), there is great mor-

tality from the disease. On the other hand, maize at a high temperature ( $24^{\circ}$ – $28^{\circ}$  C.) is hardly affected at all, but it is seriously attacked at a low temperature ( $8^{\circ}$ – $16^{\circ}$  C.). Further enquiry has shown that where wheat and maize are growing at unfavourable temperatures the nature of the outer cell walls is different from the normal, and the walls are thinner. This change in the character of the cell walls renders the plants much more susceptible to penetration by the hyphae of the fungus, and so the plants become seriously diseased. With respect to this disease, it has also been shown that both wheat and maize are affected severely at all temperatures when grown in soils of very low water-content. Here again an adverse condition for the growth of the crop plant renders it extremely susceptible.

Temperature is sometimes so unfavourable to a parasitic fungus that the latter has no chance of attacking its host. In this way the cultivated plant escapes disease, not because of any inherent property, but because the temperature is so adverse to the fungus that it cannot even initiate infection. A striking example of such an effect of temperature is seen in the distribution of the disease known as Onion Smut in the United States. This disease, which can only be established in the seedling stage, cripples the growth of the very young plants and produces unsightly black streaks of smut spores towards the base of the plant. Onion Smut is prevalent in the northern part of the United States, but is unknown in the south. Investigation has shown that the absence of the disease in the south is due to such high soil temperatures that most of the fungus spores do not germinate and are therefore incapable of causing infection (13). A temperature of  $29^{\circ}$  C. either inhibits germination of the spores or kills the delicate germ tubes if the spores do germinate.

A somewhat similar phenomenon is met with in the distribution of *Puccinia graminis* (Black Rust of cereals) in the southern United States. In this region the fungus occurs commonly on wheat, but it never affects the barberry, as it does in the north. The reason for this is that the black teleutospores formed on the wheat straw lose their vitality during the warm winters of the south and so cannot infect the barberry in the spring. In the north the cold winter preserves the life of the teleutospores and so the barberry can be infected. The same considerations probably account for the fact that barberry bushes are not infected in Australia although Black Rust is all too common there on wheat.

With the marked influence which environment exercises upon

the expression of disease-resistance it is not surprising that a variety which is practically immune to attack during one period of the year may be very susceptible at another. The variety of wheat "Little Joss" is usually very resistant to Yellow Rust, but I have seen fields of it in February and March which were yellow with this rust. When such wheat began to grow actively again in April and May the new foliage was entirely free from rust.

Some particular part of the shoot system of a plant may alone be susceptible to disease, whereas the whole of the shoots of closely related varieties may be susceptible. A variety of wheat "Norka" was recently sent to me from the United States as being very highly resistant to mildew there. One of the most critical tests for mildew-resistance is to grow the plant in a greenhouse, for if the resistance is not extremely marked mildew develops abundantly on the foliage. This variety "Norka" grown in a greenhouse remained quite free from mildew until the ears were fully developed, when the fungus developed slightly on the glumes.

In recent investigations on virus diseases of plants there has been encountered a phenomenon which in some respects is very similar to a well-known phenomenon in human pathology. With certain diseases like typhoid and cerebro-spinal meningitis some individuals can be infected with the bacteria of these diseases without becoming ill. Persons who are tolerant of these bacteria are said to be "carriers" of disease. They may, of course, be sources of infection in a healthy community.

With certain virus diseases of potatoes some varieties which appear to be perfectly healthy may be shown by suitable experiments to be "carriers" of specific viruses. By grafting one of these "carriers" on to a healthy but susceptible plant the virus is transmitted from the "carrier" into the susceptible plant, in which the characteristic symptoms of the virus disease quickly appear. At present we know very little as to the nature of virus diseases in plants, but in the varieties which are "carriers" presumably the virus is tolerated by the host or is present in it in a latent condition. In some other virus diseases of plants "carriers" have been shown to exhibit symptoms of the presence of the virus by changing the environmental conditions.

From the stress which has been laid upon the modification of disease-resistance by environmental conditions it may be concluded that the genetic quality of resistance is of little importance. That is certainly not true. The resistant quality of the variety is still



present even though it may be partly masked by the influence of an adverse environment.

Soon after the re-discovery of Mendel's Law of Heredity, Biffen (2) showed that susceptibility and resistance to Yellow Rust in wheat were definite hereditary entities transmitted in accordance with that law. On crossing a susceptible wheat with a resistant one the hybrids all proved to be susceptible; on self-pollinating these hybrids, the plants of the next generation segregated in the proportion of three susceptible to one resistant. In this example a single hereditary factor was responsible for susceptibility or resistance. Since this discovery much work has been done on the elucidation of the transmission of susceptibility or resistance to disease in plants. With regard to Yellow Rust of wheat susceptibility is dominant to resistance, but with Wheat Mildew resistance is dominant to susceptibility. The transmission of susceptibility or resistance is also sometimes more complicated than in the example outlined, and is dependent upon more than one factor. This is the case in the inheritance of susceptibility to potato Wart Disease.

With the clue afforded by the re-discovery of Mendel's law plant-breeders, the whole world over, are attempting to introduce new varieties of cultivated plants which will be more resistant to disease than those formerly grown. Many varieties of plants which are highly resistant to disease are of little commercial value because of low cropping capacity or of some other defect. Plant-breeders now often have it within their power to combine the character of disease-resistance of one variety with the heavy-cropping capacity or other desirable quality of a susceptible variety. Considerable progress has already been achieved along these lines.

It may be argued that the future control of plant diseases lies in the hands of the plant breeder. This is only partly true. Unexpected difficulties are sometimes encountered in breeding work, in which it appears impossible to combine the character of disease-resistance with the fine quality and heavy yield of susceptible varieties. Besides, all Nature being in a state of flux when any long period of time is taken into consideration, it must be remembered that pathogenic organisms themselves may change, and, with increasing virulence, may attack varieties of crop plants hitherto resistant. It is particularly the province of the plant pathologist to ascertain the conditions of growth of cultivated plants which are least favourable to attack by parasites, and to prevent disease by applying the methods of plant sanitation. The best results in the control of plant



diseases are likely to be achieved by the mutual co-operation of plant breeder and plant pathologist. With plants as with human beings we cannot foresee the time when there will be no more disease and no more death.

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## ON DESMID PLANKTON

BY BENJAMIN MILLARD GRIFFITHS, D.Sc., F.L.S.

THE normal habitat of desmids is in the water which occupies the interstices of the shoots and leaves of water-saturated vegetation, and among the vegetable debris at the base of the vegetation. Sphagnum bogs and weedy pondlets are habitats of this type. The habitat is not exactly terrestrial nor is it quite aquatic. We shall therefore refer to it as the "terraqueous habitat."

Messrs West (17) have shown that the majority of desmids occur in regions where the rock of the drainage area is hard and non-calcareous, and the water supply is deficient in salts. Pearsall (11) has shown that there is not only an absolute deficiency in salts but also a relative deficiency in salts of calcium. The pH of the water is either neutral or slightly acidic. Strongly acidic habitats are not favourable for desmid abundance, and consequently few desmids are to be found in very peaty waters of high acid reaction.

In addition to the large group of the terraqueous desmids, there is a group comprising those forms which lead a more or less free-floating existence in the watery continuum among and around the shoots of aquatic plants or out in the open water of the lacus. The free-floating or planktonic desmids fall into two classes, first the *benthoplanktonic* or littoral desmids which occur in weedy lakes, and secondly the *limnoplanktonic* or "exclusively planktonic" desmids of lakes in which the weed fringe is either very scanty or very small in relation to the volume of water (Griffiths (5)). Both these classes occupy habitats in which the salts content is low because the initial water supply is deficient in salts, but the organic content differs in the two habitats. The terraqueous habitat is relatively rich in organic matter derived from the decaying debris; in the benthoplanktonic habitat the organic matter is more diluted; and the limnoplanktonic habitat is relatively deficient in organics. There is, however, no sharp break between the watery-film terraqueous habitat and the watery-continuum habitat of semi-marshy vegetation. Bogs and weedy pondlets are in the aggregate much more extensive than actual pools or lakes. The large majority of desmids live in the former, in close association with the mosses and semi-aquatic plants of these habitats. All the habitats are deficient in salts, but they differ from each other in organic richness.

Examples of habitats are:

**Terraqueous.** Damp and boggy spots occurring over large areas in regions of ancient rock in Scotland, Cumberland and North Wales.

**Planktonic.** Benthoplanktonic; the weedy lakes of the Shetland Islands (see West and West(15); Murray and Pullar(10), vol. II, part 2) and Presaddfed Lake, Anglesey (see Griffiths(4)).

Limnoplanktonic; large bodies of still water with insignificant weed fringe, e.g. numerous Scottish and Cumbrian Lakes (see West and West(16); Messrs Pearsall(12)).

Messrs West (17), p. 204) are of the opinion that the planktonic desmids originated from the desmid community of the area surrounding the lacus, but they hold that certain limnoplanktonic desmids have become adapted to the habitat during a long period of evolution, and that these desmids constitute a peculiar alga-flora which is of ancient origin and confined exclusively to limnoplanktonic habitats.

In the following discussion, evidence will be offered to show that while it is true that the planktonic desmids originated from the terraqueous desmid flora, the "exclusively planktonic" or limnoplanktonic desmids do not constitute an ancient alga-flora. They are more probably environmental varieties which are normally present in the terraqueous desmid flora, but which only multiply when carried into the limnoplanktonic habitat. The time taken for the appearance of the "exclusively planktonic" desmids, far from being lengthy, may be as little as 40 years.

Messrs West(17) classify the planktonic desmids as follows: *P* forms which are "exclusively planktonic," i.e. limnoplanktonic, 14 species and 1 variety. *p* forms, found more often in the plankton (limnoplankton) than elsewhere, 23 species and 18 varieties. *Pv* forms, which are limnoplanktonic varieties of species of various origin, 28 varieties. Littoral forms (benthoplanktonic), 199 species and 21 varieties. Total number of planktonic desmids, 236 species and 68 varieties.

On analysing the *p* forms, it is found that the 18 varieties are made up of 7 varieties of *p* species, 5 varieties of littoral or benthoplanktonic species, and 6 varieties of desmids which are not planktonic but terraqueous. The *Pv* forms comprise 28 varieties of which



tonic" desmids, and they are considered by these authorities to be of very ancient origin, and to constitute a definite desmid group.

In the following table, the left-hand column contains the list of *P* species, together with *Micrasterias Hardyi* from the Yan Yean Reservoir, Australia (West, G. S. (14)). Reference to the volume and page of description in *British Desmidiaceae* follows each species. In the second column is given the nearest ally of the *P* desmid. The origin of the ally is given as follows: *p* = benthoplanktonic but often in the open water, *B* = benthoplanktonic, *T* = terraqueous. If the species is variable, it is marked *V*. In the last column is given the distribution of the species in Europe, Asia, Africa and America.

						Eu.	As.	Af.	Am.
1	<i>Micrasterias Murrayi</i>	2, 93	<i>M. Sol</i> (Ehr.) Kuetz.	2, 95	<i>p</i>	<i>V</i>	×		
2	<i>Cosmarium Corribense</i>	3, 120	<i>C. arctoum</i> Nordst.	3, 41	<i>T</i>	<i>V</i>	×	.	×
3	<i>C. subcontractum</i>	2, 174	<i>C. contractum</i> Kirch.	2, 170	<i>B</i>	<i>V</i>	×	×	×
4	<i>Arthrodesmus crassus</i>	4, 102	<i>Staur. jaculiferum</i> West	5, 16	<i>p</i>	<i>V</i>	×	.	×
5	<i>A. quiriferus</i>	4, 101	<i>Staur. jaculiferum</i> West	5, 16	<i>p</i>	<i>V</i>	×	.	×
6	<i>Staurostrum affine</i>	5, 128	<i>St. polymorphum</i> Bréb.	5, 125	<i>B</i>	<i>V</i>	×	×	×
7	<i>St. boreale</i>	5, 112							
8	<i>St. conspicuum</i>	4, 143	<i>St. grande</i> Bulnh.	4, 140	<i>B</i>	<i>V</i>	×	×	
9	<i>St. dorsidentiferum</i>	5, 171	<i>St. gracile</i> Ralfs.	5, 96	<i>B</i>	<i>V</i>	×	×	×
10	<i>St. ineleqans</i>	4, 153	<i>St. Clypsedra</i> Nordst.	4, 152	<i>T</i>	<i>V</i>	×	.	×
11	<i>St. pelagicum</i>	5, 124	<i>St. avicula</i> Bréb.	5, 40	<i>B</i>	<i>V</i>	×	×	×
12	<i>St. pseudopelagicum</i>	5, 107	<i>St. avicula</i> Bréb.	5, 40	<i>B</i>	<i>V</i>	×	×	×
13	<i>St. subnudibrachiatum</i>	5, 91	<i>St. brachiatum</i> Ralfs.	5, 88	<i>B</i>	<i>V</i>	×	×	×
14	<i>Desmidium occidentale</i>	5, 245	<i>D. Swartzii</i> Ag.	5, 246	<i>B</i>	<i>V</i>	×	×	×
15	<i>Micrasterias Hardyi</i>	—	<i>M. Mahabuleshwariensis</i> Hob.	2, 121	<i>T</i>	<i>V</i>	.	×	×

*Micrasterias Murrayi*. Occurs in Loch Ruar, Sutherland, and its variety *triquetra* in Loch Doon, Ayrshire (*Brit. Desm.* 2, 95). It is also recorded for Loch Bogton in Ayrshire by Murray (<sup>9</sup>), p. 302) who points out its relationship to *M. papillifera* Bréb. (see also Munster Strom (<sup>6</sup>), p. 205).

*Cosmarium Corribense*. A form obtained from pools on Mitcham Common, Surrey, is figured in *Brit. Desm.* 3, Pl. LXXV, Fig. 9. The reference is given in the description of the plate, but there is no reference in the text on p. 120. The close relationship to the ubiquitous terraqueous *C. arctoum* Nordst., and the occurrence of a *forma* in a small Surrey pool, point to this desmid being either terraqueous or benthoplanktonic, and not truly limnoplanktonic.

*Cosmarium subcontractum*. From Loch Beosetter, Shetlands, a small, very shallow and extremely weedy loch (Murray and Pullar (<sup>10</sup>), vol. 2, part 2, p. 245). The desmid was accompanied by no less than 49 benthoplanktonic species (see West and West (<sup>15</sup>)). It is evidently not limnoplanktonic.

*Arthrodesmus crassus* and *A. quiriferus*. Very closely related to *Staurastrum jaculiferum* by transitional forms (*Brit. Desm.* 4, 88 seq.). *St. jaculiferum* is a *p* species, and is variable and widely distributed.

*Staurastrum affine*. From Neugles Water and Brindister, Shetlands, where in each case it is associated with some 14 or 15 benthoplanktonic desmids. None of the Shetland Lakes are of any size or depth (Murray and Pullar(10), vol. 2, part 2, p. 231). The desmid is evidently not limnoplanktonic.

*Staurastrum boreale*. From Loch Asta, Shetlands, where it is associated with 27 benthoplanktonic species. It is very improbably limnoplanktonic.

*Staurastrum conspicuum*. Occurs in two Scotch lochs and in a small pool (*Brit. Desm.* 4, 144). Its occurrence in the latter habitat casts considerable doubt on its limnoplanktonic character.

*Staurastrum inelegans*. Of variable character (*Brit. Desm.* 4, 153). This desmid together with *St. subnudibrachiatum* and *Desmidium occidentale* occur in Loch Fadoga, Lewis, which has a mean depth of only 11 feet (Murray and Pullar(10), vol. 1, p. xxxvi). They also are doubtfully limnoplanktonic.

*Micrasterias Hardyi*. Occurs in the Yan Yean Reservoir, Australia (West(14)). West says (p. 42) that though the desmid also occurs in the littoral regions of the reservoir, its appearance there is only accidental, and that it must have been established as an exclusively planktonic species for a long time. It is, however, difficult to see the grounds for these conclusions, because the reservoir in which the desmid occurs is only 47 years old. The probabilities are that *M. Hardyi* is a form of the terraqueous *M. Mahabuleshwarensis*. West himself points out the relationship to the latter, and also shows (*Brit. Desm.* 2, 122) that the latter is a very variable species. *M. Mahabuleshwarensis* is not recorded for the reservoir, but it does occur in Australia (*Brit. Desm.* 2, 122). It is of course not possible to assert that the desmid was present in the catchment area of the reservoir, but is equally impossible to deny it. All that can be asserted is that it was not found in the particular material examined.

When an area is being searched for desmids, the actual percentage of area which is examined is usually surprisingly small. Consider a small lake 123 acres (50 hectares) in area, of which the upper layer of 3 ft. (1 metre) is being investigated with the aid of a conical plankton net 31 sq. in. in area at the mouth (200 sq. cm.). The net is towed at 4 miles per hour (6 kilom.) for half an hour.

Even making the very large assumption that the filtration is perfect, the amount of water examined is only 0.012 per cent. of the upper layer. It would also be necessary to examine the whole of the catch in order to attain even to this standard of completeness. The larger the lake the less the percentage examined. A collection made as above in Lake Windermere would only represent 0.00046 per cent. of the upper layer of water.

The percentage of the terraqueous habitat which can be examined by taking small masses of wet vegetation and squeezing out the adherent water, is almost infinitesimal. Assuming that no less than 100 samples are taken, each one square decimetre in area, then if the area examined is the same as that above, viz. 123 acres, the actual area examined is about 0.0002 per cent. of the whole, always providing that the whole of the material so obtained is submitted to examination.

Further doubt of the validity of the list of exclusively planktonic desmids is raised by their non-appearance elsewhere. None of them are recorded for the North German Plain by Donat (3); the only records for the Norwegian Mountain Lakes by Munster-Strom (6), p. 238; (7), pp. 16-18) are *Staurastrum pelagicum*, *St. pseudopelagicum*, *Arthrodesmus quiriferus*; there is no record from the Sarek Mountains of Swedish Lappland by Munster-Strom (8); the only record for West Greenland by Bachmann (1) is *Staurastrum pseudopelagicum*; in the 11 Cumbrian Lakes, Messrs Pearsall (12) record only *Arthrodesmus crassus* and *Staurastrum pseudopelagicum*; in the Wisconsin Lakes, U.S.A., G. M. Smith (13) records a form of *Arthrodesmus quiriferus*, a variety of *Staurastrum subnudibrachiatum*, and *Staur. pelagicum* and *Staur. pseudopelagicum*.

It must also be pointed out that it is notoriously difficult to determine whether a plant is a species or not, even when genetic experiment can be carried out upon it, and the cultivation of plankton desmids has not yet been achieved. In the absence of experiment, practically the sole criterion of specificity is the personal equation of the observer. Some observers appreciate similarities more than differences, and others do the reverse. The latter see new species where the former see only varieties of known species. Desmids are extremely variable organisms and there are even linkage forms between genera in many cases (Carter (2) in *Brit. Desm.* 5, 121 and also 2, 126 and 4, 89). It is of course always better in biological work to appreciate differences rather than similarities, and no observers were more careful in this respect than Messrs West. That the "exclusively

planctonic" desmids are different from the others no one can venture to deny, but it is the origination of the differences which is in question.

The appearance of an exclusively planktonic desmid in a reservoir is paralleled by the following observations. In August 1923, the writer investigated the plankton of two reservoirs both of which are situated on catchment areas of ancient rock.

Vyrnwy Reservoir, Wales, was constructed by the Liverpool Corporation in 1890 (1880-1890). It is 1121 acres in area and 90 ft. deep at the dam. The wash-line is cased in stone, and fringing vegetation is practically absent. An analysis of the water from the Vyrnwy inlet at the Prescott Reservoir on Sept. 4th, 1923, gives 3.44 parts per hundred thousand of solid matter in solution, a total hardness of 1.37, and organic ammonia 0.005. The pH of the water, taken by means of a "universal indicator" was 6.5. The water was slightly brown in colour. The plankton was scanty, and comprised: *Arthrodesmus triangularis* (p), *Staurastrum anatinum* (p), *Staurastrum pseudopelagicum* (P), *Gomphosphaeria Naegelianiana* (P), *Staurastrum lunatum* var. *planctonicum* (Pv), *Ceratium hirundinella*, 2 basal horns (P), *Dinobryon divergens* (p), *Tabellaria flocculosa*, *Spondylium papillosum*, *Chroococcus limneticus*.

West Baldwin Reservoir, Isle of Man, was constructed by the Douglas Corporation in 1905 (1900-1905). It is 42 acres in area and 75 ft. deep at the dam. There is a little fringing vegetation at the stream inlet at the top end, but not on the sides. The catchment area is moorland, lying on Manx Slates. An analysis of a water-sample taken April 17th, 1923, shows 4.8 parts per hundred thousand of solid matter, total hardness 1.0, and albuminoid ammonia 0.0045. The pH at the time of collection of the plankton was indicated as 6.5. The water was slightly brown in colour. The plankton was exceedingly scanty, and comprised: *Hyalotheca dissiliens* (B), *Arthrodesmus Incus* var. *Ralfsii* (B), *Tabellaria flocculosa* (B), *Peridinium Willei* (P), *Staurastrum anatinum* (p), *Micrasterias rotata* (B).

The following table gives an analysis of the desmid flora of the two reservoirs mentioned above and of the Yan Yean Reservoir, Australia (West, G. S. (14)):

Reservoir	Age	P	Pv	p	B
Yan Yean	47	1	2	3	6
Vyrnwy	33	1	1	2	4
West Baldwin	18	—	—	1	1

P = limnoplanktonic. Pv = limnoplanktonic varieties of other forms.

p = more often limnoplanktonic than otherwise. B = benthoplanktonic.



From the foregoing analyses and discussions, it is probable that at least eight of Messrs West's "exclusively planctonic" desmids are really either benthoplanktonic (littoral) species or varieties of variable and widely distributed benthoplanktonic or terraqueous species. There remain seven species (*Micrasterias Murrayi*, *Staurastrum dorsidentiferum*, *Micrasterias Hardyi*, *Arthrodesmus crassus*, *Arthrodesmus quiriferus*, *Staurastrum pelagicum*, *Staurastrum pseudopelagicum*) which may be either varieties of ubiquitous and variable non-limnoplanktonic species, or they may be of ancient origin and may form a definite association of plankton algae.

If the association is ancient, the original varieties must have arisen at one spot and spread from lake to lake. Messrs West, however, hold that the delicate character of the desmids and the complete absence of resistant resting stages make transport very unlikely (West and West (17), p. 206). If they have not spread they must have remained at the spots where they first appeared, and their distribution should be distinctly isolated. This is not the case, for *Micrasterias Murrayi* occurs both in Sutherland and in Ayrshire and even in Russia (? see Munster Strom (6), p. 205), and *Arthrodesmus crassus*, *A. quiriferus*, *Staurastrum pelagicum* and *St. pseudopelagicum* are distributed over different continents.

If the desmids are the products of a long evolution in the limnoplanktonic habitat, they should be found only in ancient habitats. This again is not the case, for *Micrasterias Hardyi* occurs in the Yan Yean Reservoir and *Staurastrum pseudopelagicum* in Vyrnwy, and neither body of water is 50 years of age.

It may be concluded therefore that the limnoplanktonic desmids are probably not of ancient origin. If they are recent, they are more likely to be varieties of other species than definite species themselves. If these contentions are valid, the 15 *P* species of Messrs West disappear from the table of desmids and become *Pv* varieties of benthoplanktonic and terraqueous species. The limnoplankton then becomes an assemblage of varieties derived from the great association of desmids of the weedy and water-soaked habitat.

The evidence from the three reservoirs shows that a desmid plankton may arise *de novo* whenever any body of water accumulates in a region which already possesses a desmid flora in its moist and boggy areas. The probable mode of origination of a desmid plankton may be stated as follows: In the local desmid flora are many widely spread and variable species. Among the variations are relatively rare forms which are not sufficiently adapted to the terraqueous

habitat to increase to any extent, but which are adapted for existence in the more poverty-stricken conditions of the limnoplanktonic habitat. These varieties may exist in the terraqueous habitat in the same form as they appear in the limnoplanktonic habitat, but owing to the practical difficulties of sampling an area of the terraqueous type the chances of them being found are very small indeed. It is not until the variety happens to be washed into the open water and multiplies therein that it is noticed at all. Alternatively, the variety may exist in the terraqueous habitat in a form which is not recognisably different from that of the variable type-species to which it belongs, but when it is washed into the lake it rapidly develops its potentiality, and while its terraqueous companions perish, it survives in a modified form. Its variation is originally physiological, but under the stimulus of the new habitat, differences of form arise.

In this view the desmid plankton is not in itself either ancient or definite but is a selected sample from the ancient and widespread terraqueous desmid flora. It is a relict-flora in a deficiency-habitat, and consists of those varieties which cannot compete successfully with their fellows in the normal habitat, but which can survive in the relatively poverty-stricken conditions of the open water.

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## DISTRIBUTION OF CARBON/NITROGEN RATIO IN THE VARIOUS ORGANS OF THE WHEAT PLANT AT DIFFERENT PERIODS OF ITS LIFE HISTORY

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(With 3 figures in the text.)

“THE younger the tissue, the lower the C/N ratio.” This generalisation was arrived at by a study of wheat plants during development. However, since an annual plant is metaphorically speaking all ages at once, meristem being active throughout life and constantly giving rise to new tissues of root, stem, and leaves, this rule should hold in the plant and its organs at any one time. Woo<sup>(6)</sup> concluded that the ratio of C/N varied in different plants and probably in different parts of a plant, while Kraus and Kraybill<sup>(3)</sup> qualified this by a fuller statement that in the stem nitrogen increases upward and carbon decreases upward; but gave no analytical results to support it.

A fuller investigation was made by Harvey<sup>(1)</sup> and his results led him to two generalisations:

(i) Those substances which normally decrease through the season are always most abundant in the “tip” portion and least abundant in the base.

(ii) Conversely, those substances which tend to increase during the season are less abundant in the tip and more abundant in the base.

These results he demonstrated by tables and graphs, but he took only three divisions—tip, middle, and base, and his work was done on the apple shoot alone. No investigation was carried out on the whole plant and the period covered was merely a few consecutive months.

Ribera<sup>(5)</sup>, working before Woo’s<sup>(6)</sup> time, found that lodging in wheat was decreased by cultural conditions that increase the dry weight, which, as Hedlund<sup>(2)</sup> has shown, is synonymous with increasing the carbohydrate value.

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This result was verified practically at Rothamsted in 1926 when all oats on plots containing twice the normal quantity of nitrate lodged badly, while the same variety on "normal" plots side by side stood erect.

Woo (6) considers that a high C/N value increases straw strength and decreases lodging by virtue of the amount of glucose present; or by the inducement of greater thickness of wall by increasing the development of mechanical tissue.

It was thought that a study of the mature wheat plant, and particularly of the lower nodes, would show conclusively, the rôle of the C/N relation in determining straw strength.

That the lower nodes are poor in nitrogen has been shown by LeClerc and Breazeale (4) who found that nitrogen tends to recede from the dying tips of leaves into the living portions, and as these whole leaves die it is translocated into the stem. In order to show the direction of the recession, whether upward or downward, they estimated the upper and the lower nodes and found that whether dead or alive, the upper nodes were considerably richer in this element than the lower nodes.

During the previous work it was thought that a more detailed examination of the C/N relations inside the plant at certain definite stages would serve to show the metabolic processes most active at those stages in the various organs of the plant. Starling, as the most vigorous wheat among those tested and displaying the maximum growth period, was chosen. Estimations were made simultaneously with that investigation and the methods used were the same in every detail.

The plant was studied in detail at one period during each of the three growth cycles; the Seedling cycle, the Vegetative cycle, and the Fruiting cycle. As an example of a seedling, a plant 10 days old was selected. No great detail was entered into, the chief object being the comparison of the plumule and the radicle.

Fig. 1 shows the striking contrast between plumule and radicle at this age. The plumule, as yet in its early infancy, exhibits the low C/N ratio of 3.9—almost the embryonic ratio, being rich in nitrogen with medium carbon. The radicle, on the other hand, consisting of the three primary adventitious roots shows an extraordinarily high C/N ratio. This is interesting in the light of the fact that in all Gramineae these three "primary" roots develop quickly, reaching their maximum development at just about this period and then become senescent, while absorption is carried on almost entirely

by the rapidly developing adventitious roots. Their high ratio is due in part to low nitrogen—less than half that of the plumule—showing that no attempt at storage is made by the roots and that at this early period, translocation is rapidly upward.

As an example of the plant at the height of the vegetative phase, a plant at the tenth leaf stage was chosen. This is the stage when carbon accumulation reaches its maximum. The leaves were removed and attention was confined to the stem and the last developed leaf, i.e. the tip of the plumule, as representing the chief seat of

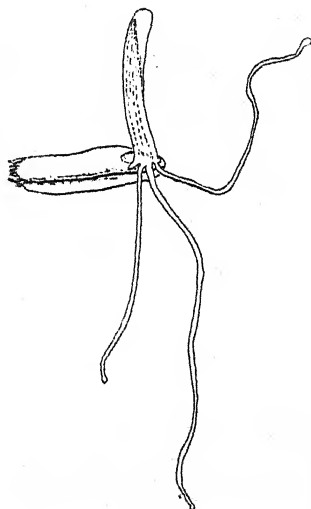


Fig. 1. Seedling 10 days old. Distribution of Carbon and Nitrogen in percentages of dry weight.

<i>Plumule.</i>	C. 29.089 %.	N. 7.423 %.	C/N 3.9.
<i>Radicle.</i>	C. 39.339 %.	N. 3.099 %.	C/N 12.5.

development at that time. This plant is represented, half natural size, in Fig. 2.

Contrary to the views of Kraus and Kraybill(3) and the results of Harvey(1) for apple shoots, in the case of the wheat there was very little range in the carbon content of the stem. Within the limits of experimental error, the carbon is practically constant throughout the entire conducting system, or at most increases by less than 1 per cent. from the roots to the base of the tenth leaf. The leaf itself contains much less carbon, showing that the products of assimilation are rapidly removed to the stem.

# Variation of C/N Ratio during Life of Wheat III

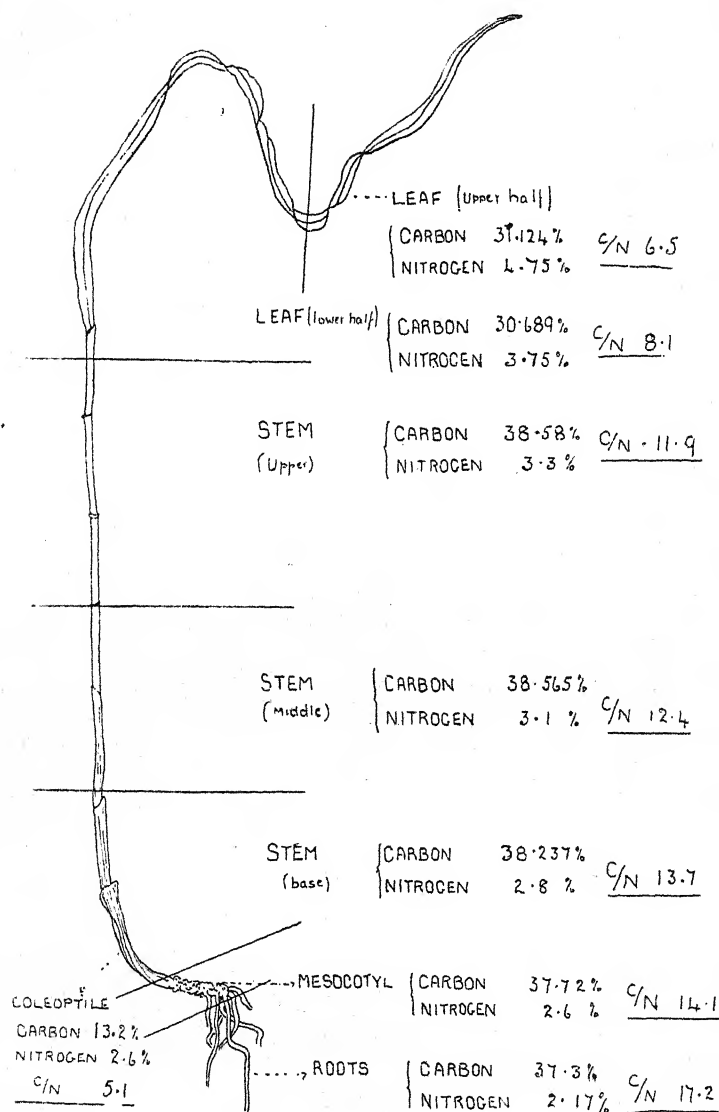


Fig. 2. Distribution of Carbon and Nitrogen in the 10th Leaf Stage, 5 months old.

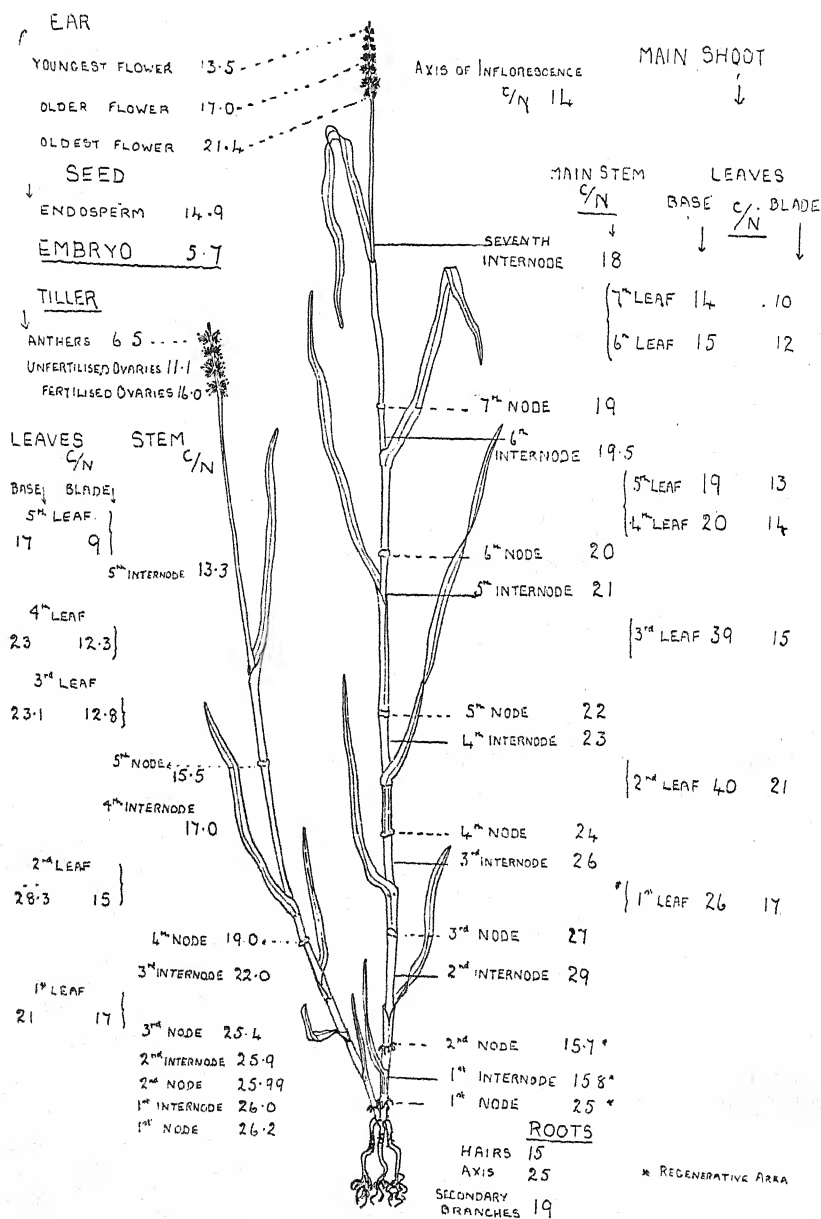


Fig. 3. Distribution of C/N Ratio on Mature Plant at Ear Stage.  
 1-10th nat. size.



## *Variation of C/N Ratio during Life of Wheat* 113

This seems to emphasise the fact that carbon accumulation is not alone responsible for flower production; if it were so, at this period it should accumulate at some portion of the stem.

The nitrogen, however, does show a marked increase up the stem as Kraus and Kraybill(3) predict. The tenth leaf is rich in nitrogen, particularly the tip which is unexpected since the actual meristematic tissue is at the base of the blade. It seems to point to a concerted upward translocation of this element probably from the root and from the older leaves at the base of the stem.

The chief significance of the results lies in the descending values of the C/N ratio up the stem. The root bases show the high value of 17.2, the mesocotyl a ratio of 14.1, while this gradually decreases until a low value of 6.5 is reached in the tenth leaf tip.

The youngest tissues have a low ratio characteristic of a plant 10 days old, the base of the stem that of a plant 110 days old. Hence, by knowing the C/N ratio of any portion, say a certain node, and by finding this value upon the curve of development (See This Journal, 27, p. 33, fig. 8) it is possible to predict the approximate "age" of that node. Conversely if we know the exact "age" of the node and are given the development curve, we may forecast roughly its C/N ratio.

Perhaps the most complete picture is that of a small plant at fully ripe ear stage. The average plant at that stage had 12 leaves but this smaller plant had only 7 leaves on its main shoot; 6 on the 3 main tillers which resembled it exactly in size and development and 5 on each of the 4 younger tillers.

Fig. 3 gives an accurate drawing of the main shoot and one secondary tiller one-tenth natural size. Only the C/N ratios are shown. For full results see Table I.

The same general results as before are shown; the carbon being constant throughout the stem, showing no localised storage of food material, the carbon values being probably those of the wood vessel walls and the sclerenchymatous tissue alone. The lower nodes were not richer in carbon than any other part, no specialised thickening having occurred there.

TABLE I.

### *Distribution of Carbon and Nitrogen. Ear Stage.*

A. The Root:			Carbon	Nitrogen	C/N
Root axis	...	...	31.929	1.292	25
Secondary roots	...	...	31.404	1.65	19
Root hairs	...	...	18.441	1.24	15

TABLE I—continued.

			Carbon	Nitrogen	C/N
<b>B. The Main Stem:</b>					
1st node	...	...	31.551	1.247	25
1st internode	...	...	31.454	1.990	15.8
2nd node	...	...	31.46	2.047	15.7
2nd internode	...	...	31.08	1.076	29
3rd node	...	...	31.0	1.147	27
3rd internode	...	...	31.0	1.200	26
4th node	...	...	31.6	1.302	24
4th internode	...	...	31.029	1.35	23
5th node	...	...	31.32	1.38	22
5th internode	...	...	30.45	1.437	21
6th node	...	...	30.45	1.57	20
6th internode	...	...	30.59	1.60	19.5
7th node	...	...	31.0	1.65	19
7th internode	...	...	31.8	1.70	18
Axis of inflorescence	...	...	31.8	2.3	14
<b>C. The "Main" Leaves:</b>					
1st leaf—base	...	...	36.67	1.414	26
blade	...	...	36.221	2.187	17
2nd leaf—base	...	...	35.737	0.887	40
blade	...	...	35.6	1.703	21
3rd leaf—base	...	...	35.9	0.9598	39
blade	...	...	35.7	2.388	15
4th leaf—base	...	...	35.7	1.886	20
blade	...	...	35.7	2.658	14
5th leaf—base	...	...	35.6	1.95	19
blade	...	...	35.2	2.70	13
6th leaf—base	...	...	35.0	2.21	15
blade	...	...	35.0	2.96	12
7th leaf—base	...	...	35.0	2.40	14
blade	...	...	35.0	3.40	10
<b>D. Tiller Stem:</b>					
1st node	...	...	32.4	1.191	26.2
1st internode	...	...	32.4	1.20	26.0
2nd node	...	...	32.4	1.219	25.99
2nd internode	...	...	31.5	1.219	25.9
3rd node	...	...	31.5	1.242	25.4
3rd internode	...	...	30.4	1.403	22
4th node	...	...	30.4	1.599	19
4th internode	...	...	30.0	1.683	17
5th node	...	...	30.0	2.021	15.5
5th internode	...	...	30.0	2.675	13.3
<b>E. Tiller Leaves:</b>					
1st leaf—base	...	...	32.737	1.577	21
blade	...	...	32.73	1.889	17
2nd leaf—base	...	...	32.73	1.215	28.3
blade	...	...	32.467	2.25	15
3rd leaf—base	...	...	32.467	1.372	23.1
blade	...	...	32.467	2.540	12.8
4th leaf—base	...	...	32.467	1.408	23.0
blade	...	...	32.46	2.641	12.3
5th leaf—base	...	...	32.4	1.90	17
blade	...	...	32.4	3.34	9

TABLE I—continued.

F. The Ear:	Carbon	Nitrogen	C/N
Flower—youngest ...	35.467	2.634	13.5
older ...	38.719	2.289	17.0
oldest ...	42.796	2.020	21.4
Stamens—young ...	40.94	7.385	5.8
old ...	31.99	6.624	6.5
Ovaries—unfertilised ...	38.36	2.54	11.1
fertilised ...	39.81	2.51	16
Seed—endosperm ...	30.7	2.18	14.9
embryo ...	49.69	8.723	5.7

The nitrogen shows the same consistent upward gradation—there is no distinction in nitrogen content between node and inter-node—the graphical representation would be an unbroken ascending line. However, it will be seen that the two lowest nodes of the main stem and the lowest node of the secondary shoot are giving rise to adventitious prop roots, and the whole region enclosed by them has become a regenerative centre. It is therefore assumed that a high nitrogen content, lowering the C/N ratio, is effective in initiating regenerative germination.

Thus the supposition that a low nitrogen content makes for straw strength by increasing the ratio of C/N in the lower nodes finds no support from this investigation. The fact of the weakness of the straw in heavily nitrated plots rather has its explanation in the theory that normally all the available nitrogen is used up to balance the accumulating carbon to produce vegetative growth. In the wheat there is no storage of available carbohydrate reserve in the vegetative tissues, the excess carbon going to the mechanical tissue. If the nitrogen is above the average, more of the carbon is used up for growth, and greater vegetative development occurs at the expense of the laying down of mechanical tissue, not at the nodes only, but throughout the entire stem. This is clearly demonstrated by the prematurely "woody" nature of nitrogen starved plants.

The C/N ratio again shows the upward decrease in the stems and in the leaves. The flower paleae also show an increase in ratio with increase of age, while the lowest ratio in the whole plant is found in the embryo, which is potentially the most highly metabolic part present.

A consideration of these figures leads to a modification of Harvey's(1) generalisations. It is true that nitrogen tends to decrease throughout the growing season, and is more abundant in the tips and less abundant in the base, yet carbon which fluctuates throughout the year shows very little marked gradation.

It upholds the finding of LeClerc and Breazeale<sup>(4)</sup> in showing the upward translocation of nitrogen from the dying to the living tissues, although I am rather inclined to interchange cause and effect and to suppose that the nitrogen is deliberately withdrawn from the leaves into the developing ovaries and embryos, and death thus follows as a result of the greatly increased C/N ratio (as in Kraus and Kraybill's Class IV) which is inhibitory of both growth and reproduction.

The C/N ratio, however, shows a distinct upward decrease unless any particular portion of the plant is rejuvenated. Apart from this, a study of the composition of an annual plant at any time during its growing period upholds the statement—"the younger the tissue, the lower the C/N ratio."

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## ON AN ABNORMALITY IN *DIGITALIS PURPUREA*

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(With 6 figures in the text.)

A CURIOUS malformation in one of the flowers on a raceme of *Digitalis purpurea* was observed during the past summer by Dr T. G. Hill in a specimen occurring in his garden, but most probably derived from wild seed; and in view of the exceptional nature of the abnormality it was felt that the occurrence ought to be placed on record.

Various types of teratological flower forms have been recorded in the Foxglove, and both synanthly and peloria (1, 2) are common; the latter most often affects the flower at the summit of the inflorescence and may be associated with fasciation. Various abnormalities are also known in the androecium, and the inheritance of a heptandrous condition in which the corolla was absent has been worked out by Saunders (3).

The present record is concerned with the lowermost flower in an inflorescence of *D. purpurea* which was otherwise normal in every respect; the malformation consisted essentially in a proliferation of this lateral floral axis and was attended by several minor abnormalities. The peduncle of the flower in question, the basal one of the inflorescence, arose normally in the axil of a leafy bract, and at a distance of some 3 cm. bore the flower which was not only abnormal in every floral whorl, but which was proliferated, giving rise to another inflorescence axis; on this the flower buds were arranged in a clockwise spiral around the axis. There was no apparent tendency for all the buds to be turned to one side, although the older buds had reached a stage of development at which this could be detected in a normal specimen.

A general view of the monstrosity as seen from the side is given in Fig. 1, in which one well-developed flower bud (*B*) is seen arising from the axil of one of the sepals, and another (*E*) is borne on the proliferated axis above the flower. Within the corolla, the axis bears a large number of flower buds. An examination of the specimen

(for diagram see Fig. 2) showed the calyx to include three normal sepals, of which two were the anterior ones and the other a lateral member of the whorl. The opposite lateral sepal (Fig. 1, C, Fig. 2, B) was normal at the base, but it was prolonged to twice the usual length, the distal portion being petaloid. This structure bore a flower bud (Fig. 1, B) in its axil. The posterior sepal was reduced to less than half the normal size and was slightly displaced in the downward direction on the peduncle. In its axil was a tiny bud



Fig. 1. View of specimen as seen from the side. A, small bud in axil of posterior sepal. B, well-developed flower bud arising in axil of petaloid sepal C. D, the new inflorescence axis produced within the flower and bearing many flower buds, of which E is one.

(Figs. 1 and 2, A) which contained the rudiments of another flower, but which was so immature that it was not possible to determine whether or not it was in any way abnormal.

The corolla was petaloid, and showed the usual characteristic coloration and markings of the species; but it was split between the two posterior components, the two edges thus formed being fused in their basal portions with the posterior sides of the proliferated axis (Fig. 2, D) which was produced forwards within the remaining part of the corolla. It would thus be possible to regard this new

axis as a development arising in the axil of the posterior sepal, but as this already carried one bud (Fig. 2, *A*) such an interpretation would involve the postulation of an accessory bud—and the occurrence of two buds in the axil of a sepal is difficult to accredit.

The corolla was perhaps chiefly remarkable for the possession of a spur-like outgrowth in the median plane of the anterior petal. This "spur" was well marked and conspicuous, being over 1 cm. in

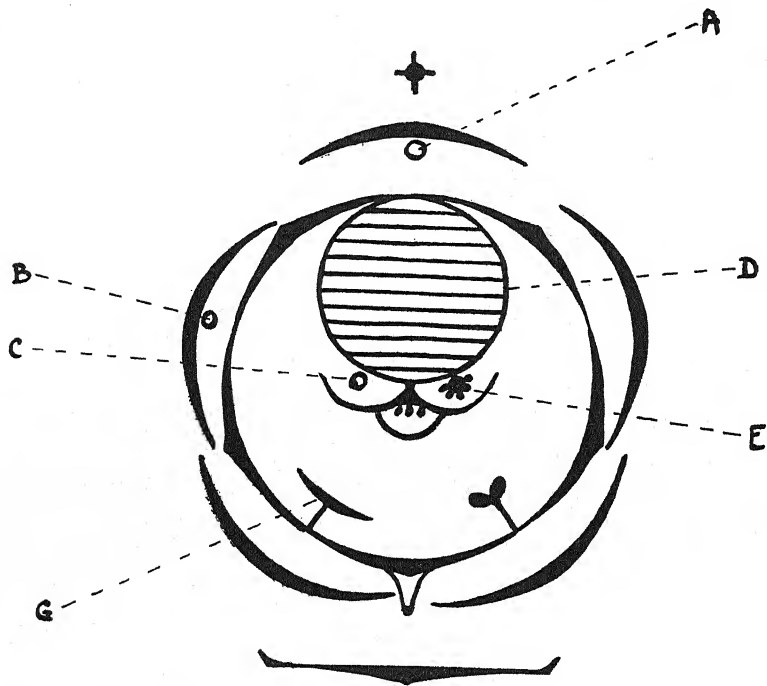


Fig. 2. General diagram of the specimen. *A*, immature flower bud in axil of posterior sepal. *B*, bud in axil of lateral petaloid sepal. *C*, flower bud in axil of leaf contributing to the formation of the gynoecium. *D*, proliferated axis bearing many flower buds. *E*, "placenta" in axis of open carpellary leaf. *G*, petaloid stamen.

length; it tapered distally but produced neither honey nor glandular tissue.

Of the four stamens normally occurring in this species only two were represented, and these were the anterior ones. Elsewhere in this family when there are normally only two stamens, as in *Veronica*, the tendency is to suppress the posterior stamen and the anterior pair, but in the present teratological specimen all three posterior

stamens were absent. Only one of the two stamens represented was normal and functional; the other was entirely lacking in any indication of differentiation into anther or filament; it was simply an elongated and free petaloid member showing the characteristic markings and texture of the corolla to which it was fused at the base.

In the position of the gynoecium were two green and leaf-like structures arising from the proliferated axis of the flower, but fused in the anterior median plane below the new axis. One of these structures bore a flower bud in its axil (Fig. 2, C), the other had, in a similar position, a short solid outgrowth bearing ovules on its abaxial surface, and somewhat suggestive of the axile placenta of normal forms. The line of fusion between the two carpellary leaves was somewhat thickened and was prolonged distally into a structure which produced two forks reminiscent of the normal stigma but of more massive proportions and without any stigmatic surface. Below the line of fusion of these two carpellary leaves, and confined to the more basal portion were attached a number of ovules; these were enclosed on the anterior side by a leafy growth which was completely fused at each side with the other two carpellary leaves—thus forming a loculus which might be taken to correspond to the anterior of the two loculi of the normal gynoecium. This anterior carpellary wall could, in the present specimen be taken to represent a leaf arising from the floral axis, enclosing in its axil a placenta apparently formed by the fusion of its own margins with those of the two neighbouring leaf-like structures contributing to this abnormal gynoecium. The ovules appeared to be normal both with regard to external form and internal structure.

An examination of the flower buds arising on the proliferated floral axis showed some of them to be abnormal. The first (Figs. 1 and 2, A) was at such an early stage of development that it was not possible to detect any abnormalities. The next in order of origin was that arising in the axil of the lateral petaloid sepal of the original flower. This bud (Figs. 1 and 2, B, also Fig. 3) possessed a normal calyx, and a corolla in which the normal zygomorphic features were present, but were not very strongly marked. The chief feature of interest in this bud was the presence of five perfect and regular stamens of equal length, such as might occur in a peloric flower. The gynoecium was normal except that the style was rather short.

The next bud, the third on the new axis (Fig. 1, E, also Fig. 4), was that arising in the axil of a green and leafy bract on the side of the axis opposite to that occupied by the flower, that is, above it.



This flower bud showed only a very slight abnormality, and except for the fusion of the two anterior sepals it was quite normal.

A flower bud with a distinctive structure was that arising in the axil of one of those leafy bracts associated with the gynoeceium of the original flower (Fig. 2, C and Fig. 5). Here the posterior sepal

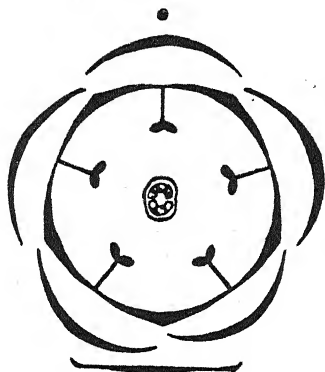


Fig. 3.

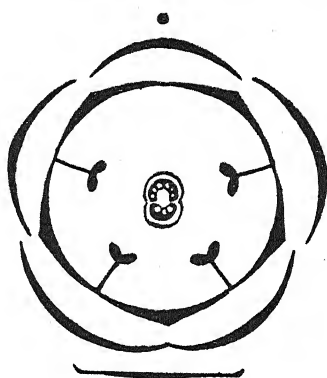


Fig. 4.

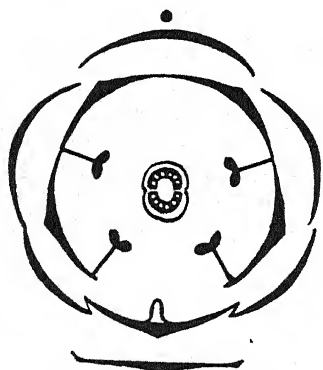


Fig. 5.

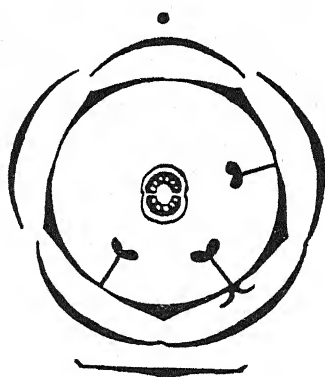


Fig. 6.

Fig. 3. Diagram of second flower bud (Fig. 1, B).

Fig. 4. Diagram of third flower bud (Fig. 2, C).

Fig. 5. Diagram of fourth flower bud.

Fig. 6. Diagram of fifth flower bud.

and one adjacent to it were normal; the others were fused together, and in the anterior median plane were apparently also fused with the anterior corolla member. The median portion of this composite structure was petaloid and it carried a small but definitely marked enation, the "spur" being directed inwards and backwards towards

the axis, and the opening being external. The remainder of the corolla which was quite separate from the anterior fused structure, formed a hood in the posterior and lateral positions on which distally could be distinguished the lobing normal on that part of the corolla. The stamens were four in number, apparently normal, and attached to the base of the hooded portion of the corolla. The gynoecium was also normal.

In the fifth bud on the new axis (Fig. 6) the posterior portion of the calyx was normal, but one of the anterior sepals was petaloid, and was fused with the sepals on either side of it. The corolla was normal except in a position immediately inside the petaloid sepal, where two free phalanges were developed on the outside. These may have represented an early fission between the two petals in that position, which was subsequently healed by a fusion occurring parallel to the cut margins but a little away from them, thus leaving them free on the outside. The androecium consisted of three stamens only, the missing member being one of the lateral posterior ones, on the side opposite to that on which the other abnormalities occurred. The gynoecium was normal.

The sixth flower bud arising on the new inflorescence axis was in structure essentially the same as the fourth (see Fig. 5). It exhibited the same fusion of calyx members, with the anterior member of the corolla, the floral envelope in this region consisting of a single composite structure. The absence of an enation in the younger bud was the only respect in which the structure of the two buds differed.

More distally the proliferated axis bore a large number of other flower buds, all of which arose in the axils of leafy bracts: the seven immediately above that last described were sufficiently developed for their structure to be discerned with a hand lens, and they all appeared to be quite normal. The remaining buds were probably also normal, but they were too immature for any definite verdict to be given.

The outstanding problem raised by the specimen is the status of the proliferated axis. The presence of five sepals, a complete though divided corolla, and two carpels, suggests that the origin of the new structure might be in the staminal whorl. This view obtains further support from a consideration of the new structure in relation to the gynoecium of the original flower. The lower (anterior) carpel remains comparatively unaltered and aloof, but the upper one is an open leafy structure arising apparently on the new axis and bearing a placenta in its axil. It would therefore appear that the proliferation has occurred posterior but closely adjacent to the gynoecium.

It has been mentioned above that a possible interpretation would regard the new axis as arising from a second bud in the axil of the posterior sepal, but on the whole its point of origin would seem to be within the corolla.

In addition to the lack of a posterior stamen normal in the genus, the present specimen showed a suppression of the adjacent pair of stamens, unless these could be traced in the green and leafy structures arising further up on the new axis, and bearing flower buds in their axils. The posterior stamen might perhaps be similarly represented.

Since one stamen is normally suppressed it is in accordance with the tendencies exhibited elsewhere in this family to postulate the suppression of all three posterior stamens; one is therefore led to suggest that the new inflorescence axis represents either a bud in the axil of the suppressed posterior stamen, or the combined product of the metamorphosed stamens themselves.

It must be noted that the specimen provides a long series in the suppression of stamens; thus the bud represented in Fig. 3 contained five stamens, several buds had the usual complement of four, and that portrayed in Fig. 6 had three. In the original flower only two stamens were represented, and of these only one was functional, the other being completely petaloid.

The presence of a "spur" on the corolla of the original flower is of considerable interest, since it suggests an affinity with other zygomorphic genera of this family, more especially *Linaria*; and to this suggestion considerable weight must be added by the fact that such spur-like tubes have previously been recorded both in *Digitalis purpurea* and *Antirrhinum majus* (4). The danger of stressing unduly the value of evidence of affinity afforded by teratological specimens is, however, well known; and in the present instance the fact that the "spur" was non-functional must detract considerably from its importance. Further, in one of the buds on the proliferated axis (Fig. 5) was an enation in a similar position, and in each case the abnormality may simply represent an expression of superfluous vitality producing an extra growth at a place of weakness.

In conclusion I should like to express my thanks to Dr T. G. Hill not only for his kindness in providing me with the specimen but also for his helpful criticism and advice.

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## REVIEW

*Enzymes. Properties, Distribution, Methods and Applications.* By SELMAN A. WAKSMAN, M.S., Ph.D. and WILBURT C. DAVISON, M.A., M.D. London: Bailliere, Tindall and Cox, 1926. Pp. xii + 264. Price 25s. net.

To undertake a work of fairly moderate compass which shall present a summary of information now available in regard to enzymes requires some courage when it is considered what a vast and scattered literature has to be digested for such an undertaking. We have to thank Professors Waksman and Davison not only for attempting this task, but for presenting us with a coherent account of enzyme activity which cannot fail to be of the greatest service to all biologists and biochemists.

The scope of the work may be indicated by reference to the main divisions into which it is divided. The titles of these divisions are: "Properties of Enzymes," "Distribution of Enzymes," "Methods of Preparation and Study of Enzymes" and "Practical Applications of Enzyme Activity."

In the first of these sections separate chapters are devoted to the part played by enzymes in biological processes, the chemistry of enzymes and to the factors which influence enzyme reactions. It might be urged that a relatively larger portion of the whole work might have been devoted to these more general aspects of enzyme study, several important questions being dealt with very briefly. Thus, the question of combination of enzyme with substrate is disposed of in fourteen lines.

The section dealing with distribution of enzymes opens with the rather startling statement: "Life is just one enzyme reaction after another." Most plant physiologists would probably be prepared to deny the truth of this, and in any event it is a crudity which, in the opinion of the reviewer, is out of place in a scientific text-book. The section is divided into three chapters dealing respectively with enzymes in the animal body, in the plant and in micro-organisms. It is the chapter on plant enzymes which will afford most interest to readers of this journal. There is first a brief survey of the distribution of the various enzymes in plants and then a summary of the enzymes found in various plant organs, namely, dormant and germinating seeds, leaves, pollen and roots. It may here again be urged that the treatment errs on the side of brevity.

The third section of the book, that which deals with the preparation and study of enzymes, will in all probability prove the most valuable part of it. It comprises nearly half the whole text and contains a mass of detailed information with regard to the modes of preparation and the properties of the individual enzymes that must be of the greatest usefulness to the animal physiologist, the plant physiologist and the biochemist alike.

The brief fourth section on the uses of enzymes forms an interesting conclusion to the book, and although short, is sufficient to bring out the important industrial applications of a knowledge of enzyme activity.

The bibliography of 1323 references, the compilation of which must have been a work in itself, adds greatly to the value of the book. The authors are to be congratulated on the production of a work which will render the task of student, teacher and researcher very much easier.

W. STILES.

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## THE ROOT AS AN ABSORBING ORGAN

### I. A RECONSIDERATION OF THE ENTRY OF WATER AND SALTS IN THE ABSORBING REGION

By LORNA I. SCOTT AND J. H. PRIESTLEY.

(With Plate II.)

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#### INTRODUCTION

CERTAIN prevalent conceptions of the root, in relation to its function of absorption, seem to require re-examination and it is the object of the present paper to reconsider the question of the entry of water and salts in the absorbing region of the root in the light of recent anatomical studies.

Coupin (4) has suggested that the main absorbing area of the root is the mass of meristem which is embedded just behind the root cap. The experimental evidence for this view has been shown to be quite inadequate (21) and the assumption that water and salts pass freely across a mass of dense, unvacuolated protoplasts does not seem to require further examination. Kroemer's (12) work upon the nature of suberised walls makes it clear that water and water-soluble substances must enter the root below the region where the walls of either endodermal or exodermal cells become completely suberised, —a region which may or may not be covered with root hairs. Further it has been pointed out by Kroemer (12), Mylius (15) and Plaut (17) that the distribution of tissues with suberised membranes varies with

the season. But apart from the general assumption that the area of the root commencing behind the growing point is the absorbing region, and that an area farther back, which may be covered with cork, has no absorbing function, little attempt is usually made to utilise the information that can be acquired by anatomical study to define the absorbing surfaces.

Reference to the literature will show that exchange of water and solutes between soil and root is usually attributed almost entirely to the root hairs, although a critical examination of the subject will show this assumption to be most insecurely based. The general acceptance of this view may be illustrated by the following selection of quotations:

1. "The greatest interest attaches to the hairs themselves, since these structures are almost entirely responsible for the functions which are ascribed to the whole absorbing system" ((7) p. 219).

2. "The actual or functional importance of permeability is perhaps greatest in the plant in the root hair action... All exchanges between the soil and the medium must take place through these cells" ((14) p. 1).

3. "Apart from such exceptional conditions, we may designate the root hairs as the special organs for the absorption of water" ((11) p. 28).

4. "All the materials that enter the plant from the soil must first pass through the walls of the root hairs or other epidermal cells as well as the layer of protoplasm lining them" ((28) p. 43).

In connection with the conclusions drawn in this paper as to the absorbing function of the various root surfaces, a certain amount of experimental work falls to be discussed, but in many cases the anatomical and microchemical data can alone be quoted as evidence. It may be emphasised, however, that the traditional view, that the root hair is the essential organ of absorption, has a very scanty experimental basis.

It must be emphasised that the entry of water and of salts into the plant presents two independent problems and recent American work has gone far to support a conclusion that physico-chemical considerations alone would render highly probable. There is no excuse for the old fallacy that the rapid intake of water and its subsequent evaporation from the plant necessitates a correspondingly high intake of salts drawn in with the water. In so far as the salts concerned are water soluble, the anatomical data as to barriers against the entry of water are obviously of significance, but the

possibility of water entry by osmosis leaves the problem of the diffusion inwards of salts still unsolved.

#### THE STRUCTURE OF THE ABSORBING REGION OF THE ROOT

Just behind the apical meristem, the cells of the root vacuolate, piliferous layer and cortex differentiating to the outside and stele to the inside. Contemporaneously with the vacuolation of the cells, the nature of the cell wall undergoes a change and passes through a stage transitional between the condition of the walls of the meristem impregnated with protein and the typical cellulose walls of the mature cortical cells. In this transitional stage, the walls give a blue colour with iodine in potassium iodide alone, or after a short treatment (about 15 mins.) in Eau de Javelle to remove residual protein, without the further addition of a hydrolysing agent as in the usual tests for cellulose. This reaction occurs in a region where the cells are undergoing rapid extension, and Ziegenspeck (30, 31), noting the extensibility without elasticity of the wall, which he compares with moist parchment, terms it the "amyloid stage" in the differentiation of cellulose. He finds a wide distribution of the amyloid stage in many young extending tissues of different plants (31). Following the root tissues farther back from the meristem, the cells gradually lose their power of extension and the cells cease to give the amyloid reaction. It may very easily be seen, however, that all the root tissues do not vacuolate simultaneously at the same distance behind the meristem, but that differentiating cells appear first in the region of the middle and inner cortex and are particularly late in doing so in the peripheral layers of the root and the peripheral layers of the stele; this fact will obviously influence the final type of tissue produced in these regions. For example, at the time the cortical cells are extending, they are enclosed between tissues which are relatively more meristematic and which at first keep pace with the extension by continued cell division and then by the early stages of their own extension also. As a result the middle and inner cortical cells extend freely, and being turgid tend to round off from one another, so that air spaces are developed in the angles between the cells. On the other hand, when the piliferous and endodermal layers reach the stage of maximum extension, the cortical cells have almost ceased to extend, with the result that the piliferous and endodermal cells differentiate closely pressed to one another and no air spaces are formed between the cells.

When the piliferous layer reaches the stage of extension, its cells are under rather different conditions from those of the internal layers, in that they are in contact with other cells on their inner and lateral faces, but the outer wall is exposed to air. As the result of the development of internal hydrostatic pressure at a time when the wall, in contact with air, is in the extensible, amyloid stage, the cells tend to extend in the direction of least resistance and root hairs are developed. Root hairs are very variable in their occurrence in different plants under different conditions, but as a general rule they are best developed when the piliferous cells are well aerated, and do not arise when the roots are actually in contact with water. As exceptions to this rule, Farr(6) gives a list of plants which still produce root hairs under water culture conditions. On the other hand, *Citrus* is a plant which frequently fails to develop root hairs, but in which they are developed under relatively dry conditions.

The wall of the root hair remains in the extensible stage, and continues to give the amyloid reaction, longest at the apex, as shown by Ziegenspeck(31). This agrees with the long recognised fact of the apical growth of root hairs, which was demonstrated by Zacharias(29) by staining the root of *Lepidium* in a solution of Congo Red and then watching the way in which the depth of staining decreased as the result of growth in moist air.

Behind the region of maximum extension, the walls of the piliferous layer and cortex gradually become completely differentiated into typical cellulose, and consequently the cortex in this region may be considered as a sponge of cellulose walls which encloses in its meshes the protoplasts of the cortical cells. This system of cellulose walls extends inwards from the surface of the root as far as, and including, the outer tangential walls of the endodermal cells, but comes to an end with the radial and transverse walls of the endodermal cells which are impregnated with the fatty substances of the Casparian strip(20). The protoplasts of the cells are firmly embedded in the Casparian strip, so that the endodermis forms a continuous cylinder, which effectively separates the cortical tissues from those of the stele.

After a time, varying with external conditions, the longitudinal extension of the cells of the root comes to a complete standstill, but they may pass through certain later stages of differentiation. In so far as these changes affect the structure of the root as an absorbing system, they will be considered in a second paper.



## THE ABSORBING REGION IN RELATION TO THE ENTRY OF WATER

Under normal conditions, an absorbing root system, of the general structure just considered, lives in the soil from which it draws water and inorganic salts. If one considers first, the root system in culture solution or in a soil in which the water is free to move, the superficial cellulose walls of root hairs and other epidermal cells will be in contact with the external solution and there is no reason to assume any obstacle to the free diffusion inwards of this solution along the cellulose walls of the cortex up to, and including, the outer tangential walls of the endodermis. The cortical and the endodermal protoplasts will all be in contact with this solution and will take up water until they become fully turgid. At the endodermis, further inward diffusion by way of the walls is stopped by the relatively impermeable network of the Casparian strip.

In the stele, on the other hand, the solution diffusing in the cellulose walls of the tissues, is a solution continuous with that in the vascular system of the plant since no semipermeable protoplasts clothe the lumina of the xylem elements. Consequently the endodermal protoplasts are in the peculiar position of being in contact on the outer side with the soil solution, and on the inner side with the stelar solution. Since the endodermal cells are fully turgid, the endodermis may be considered as a single, semipermeable membrane, separating the two solutions, and water will be drawn across it by osmosis, either inwards or outwards, according to which solution is of the higher osmotic strength. The osmotic concentration of the external solution is readily determined and is usually relatively dilute. Some idea of the concentration of the stelar solution may be obtained from the sap exuded by cut stumps of growing plants and especial interest attaches to the results obtained by Litwinow (18). He showed that the exudation sap has an osmotic pressure 0.5 to 2.0 atmospheres higher than the external solution and that with alterations of the osmotic pressure of the external solution, the concentration of the stelar sap also becomes adjusted, so that the difference is maintained. According to these results the root will draw in water across the endodermis with an osmotic pull equal to 0.5 to 2.0 atmospheres. The maintenance of this internal concentration requires a constant supply of solutes and these are available in the growing root as the result of the continual process of differentiation. As a new cell is added to the xylem chain by this process, it loses its semipermeability and its soluble contents are added to the solution.

From these considerations, one comes to the conclusion that under conditions of plentiful water supply, the walls of the tissues external to the endodermis are permeated with the soil solution, whilst those internal to the endodermis are permeated with stelar solution. These two solutions are separated by the semipermeable protoplasts of the endodermis and water is drawn into the stele by virtue of the higher osmotic concentration of the contained solution. Thus the functional absorbing surface of the root is the endodermal surface, and under these conditions it is quite immaterial whether the actual surface of the root is increased by the production of root hairs, or not. This view also avoids the necessity of assuming a gradient of osmotic pressures across the cortex in order to explain root pressure<sup>(19)</sup>, an assumption which is in conflict with the results of Ursprung and Blum<sup>(25)</sup> on the suction pressures of the surface cells of roots growing in water or solutions. They find that these cells have no suction pressure and that this remains true when the concentration of the external solution is altered.

In the foregoing discussion it was stated that the cells external to the endodermis would all be in contact with the soil solution, percolating in the cellulose walls and intercellular spaces of the cortex, and they would naturally take up water until the internal osmotic pressure was balanced by the back pressure of the wall. The cells would then be fully turgid and there would be no residual osmotic pressure tending to draw water into the cell—i.e. suction pressure. The whole process just described would appear to fit the facts so long as the root is in water culture or in a soil with a sufficiently high water content for the water to be free to move, but the question needs some further consideration in relation to different degrees of soil moisture.

In such complex systems as soils, it is not possible to establish a definite table of classification of soil moisture, but certain soil constants have been defined, which serve for the comparison of the water-holding capacities of different soils. These soil constants, arranged in order, provide a few points on a rough scale of soil moisture:

*Maximum water capacity.* The water fills the entire pore space as in a water-logged soil.

*Moisture holding capacity or maximum field capacity.* The water retained is the maximum amount that the soil can hold against gravity drainage.

*Moisture equivalent.* Water held against a centrifugal force of 1000 gravity. (Briggs and McLane<sup>(2)</sup>.)

*Wilting coefficient.* Water content of the soil at which plants wilt permanently. (Briggs and Shantz(3).)

*Hygroscopic coefficient.* Water held in equilibrium with a standard concentration of sulphuric acid (usually 10 per cent.).

The values picked out on this scale are arrived at by different methods of estimation and merely give particular points on a continuous scale. In criticising Bouyoucos'(1) system of classification of soil moisture, Parker(16) emphasises the fact that the drying of a soil is a continuous process, in which, as the moisture content decreases, the attraction between the soil and the remaining water increases. Certain values of this force holding the water emerge by special methods of investigation(22), but do not represent points at which the water is held in a different way in the soil.

As one passes down the scale and the water content decreases, the remaining water is more firmly held, and its power of free movement in the soil gradually falls off. The question that arises in relation to the plant is—at what moisture content of the soil will the plant remove water more rapidly than the supply can be maintained by movement of water from particle to particle in the soil? And on this question the scale of soil moisture gives no definite information.

Veihmeyer(27) emphasises the fact that when a definite amount of water is added to the surface of a soil, the soil is wetted to a definite depth up to its maximum field capacity. This movement of water will be by gravity flow and is a relatively rapid process, 50 per cent. of the water movement which takes place in five days occurring in the first hour. The further distribution of the water to the deeper layers by capillary creep is so slow as to be negligible in comparison with the rate of removal of water by the plant. As regards the upward movement of water in soils by capillarity, he refers to Alway in America and Rotmistrov in Odessa, who independently come to the conclusion that water which has sunk to a depth of about 1 ft. in the soil, never reaches the surface again except through the agency of the plant. As the result of the very slow movement of water in soils which have a moisture content below the maximum field capacity, there is a tendency for the growth of plants to cause an uneven distribution of water, since the soil immediately in contact with the roots gives up its water to the plant more rapidly than the supply is replenished by movement of the water in the soil. Briggs and Shantz(3) point out that these facts need taking into consideration when estimating the wilting coefficients of soils, for if the soil in contact with roots is drier than the soil not in contact,

the value of the wilting coefficient will vary with the type of root system of the wilted plant. For example, the fine fibrous rooted grasses give the wilting coefficient of a soil at a lower moisture content than that obtained by using a coarse rooted plant such as *Colocasia*.

At moisture contents of the soil which do not fall far below the maximum field capacity, although the movement of the water in the soil is relatively slow, the water is not very firmly held by the soil and is readily withdrawn by the plant so long as the root is continually coming into contact with new soil particles by virtue of growth and the development of new root hairs. On this point Viehmeyer refers to the conclusion of Burr that the roots of plants must extend themselves into the soil where available water is present, rather than depend upon the water being brought to them by capillarity.

At lower moisture contents the movement of water in the soil becomes a still more negligible factor, and also the water will be more firmly held by the soil particles and the rate of exchange between the soil and the root will fall off proportionately. The functional absorbing surface of the root is still the endodermal surface and if entry across this layer is to be maintained above a certain rate, it is clear that there will be a minimum rate at which exchange between the soil and the root surface must take place in order to maintain a sufficient supply of water to the plant. But the external root surface is large in comparison with the endodermal surface and the rate of exchange between soil and root surface can be slower than the rate of entry across the endodermis in proportion to the extent that root surface exceeds endodermal surface. At the lower moisture contents of the soil, therefore, the increase in area of the root surface by the development of root hairs acquires an importance, since by them contact is made with a larger volume of soil and a sufficient supply of water to the plant may be maintained by virtue of the increased surface, even when the actual rate of withdrawal of water from the soil particles is relatively slow.

It is interesting to find the point at which plants wilt permanently included in a table of soil constants, but that this is a constant of the soil rather than of the plant is evidenced by the fact that the value for a soil is practically the same, regardless of the plant with which it is determined (but see the exception cited above), and the value may be determined with a fair degree of accuracy by dividing the moisture content of the soil at the moisture equivalent by 1.84

(Briggs and Shantz(3)). Evidently, at this value of the moisture content, the rate of exchange between the soil and the root surface falls below the minimum rate required in order to maintain a sufficient rate of exchange across the endodermis. The failure lies in the slow rate of water movement in the soil, which is determined by soil structure and composition, and so we see the wilting coefficient used as a soil constant.

Under conditions of excess supply the water diffuses freely along the cellulose walls of the cortex, and consequently the cortical and endodermal cells take up water until they are fully turgid and have no further suction pressure. The only osmotic exchange which persists is that which draws water across the endodermis. As the moisture content of the soil approaches the wilting coefficient, the rate at which water is drawn in across the endodermis tends to exceed the rate of entry at the surface of the root and water will be drawn from the endodermal cells to maintain the supply. As the cells lose turgidity, they develop a suction pressure and withdraw water from the next external cells and so a gradient of suction pressures is established across the cortex, extending from the endodermis to the superficial cells of the root. Ursprung and Blum(24-26) have measured the suction pressures of root cells of plants growing in sawdust and find that there exists such a gradient across the cortex. Under these conditions some water will be drawn from the soil by the suction pressure of the surface cells, and this will be drawn in across the cortex by virtue of the rising suction pressures of successive cells.

As far as results show, no fixed value for the soil moisture can be determined at which the plant ceases to remove water from the soil, and the value at which the plant cannot withdraw sufficient water to maintain its vitality will depend to a considerable extent upon the external conditions affecting the rate of transpiration. The wilting coefficient is only of value as a soil constant when wilting is brought about gradually and not as the result of rapid transpiration. When this is the case the value tends to be too high. Sometimes plants are able to reduce the soil almost to the moisture content of the hygroscopic coefficient, and Shull(22) has shown that *Xanthium* seeds can reduce the moisture content to about the same extent, so long as good contact is maintained by shaking the soil and seeds together.

Bouyoucos is probably correct in suggesting, however, that at these low moisture contents the amount of water taken up by the

plant is important in maintaining vitality but does not enable growth to take place. If growth ceases, the differentiation of new xylem elements will also stop and there will be little addition to the solutes in the xylem sap, with the result that the osmotic pull of the stelar sap will fall off. There is still the possibility that water may be drawn into the stele by the tension set up in the vascular tracts by the transpiration pull, and the cohesion theory, as usually stated, assumes that this tension is responsible for the intake of water into the plant. When growth ceases, however, the later stages of differentiation still continue and the microchemical changes in the walls which take place, and which will be discussed in a second paper, may render the continued intake of water of short duration.

#### THE ABSORBING REGION IN RELATION TO THE ENTRY OF SALTS

In the previous consideration of the absorbing region of the root in relation to the entry of water, it has been shown that there is every reason to suppose that the soil solution diffuses freely along the walls of the cortex, and that water is then drawn across the endodermis by osmosis. This accounts only for the passage of the solvent across the semipermeable protoplasts of the endodermis and cannot explain the entry of dissolved salts.

De Lavison (5), working on the entry of solutes into the root, came to the conclusion that the substances investigated by him fell into two groups:

(a) Substances capable of penetrating protoplasm, which readily diffuse across the cortex and across the endodermis.

(b) Substances incapable of penetrating protoplasm, which diffuse along the cellulose walls of the cortex, but are stopped at the endodermis.

As an example of group (b), De Lavison found that iron in very dilute, non-toxic concentrations could be traced by means of microchemical tests along the cortical walls, but appeared to be completely stopped at the Casparian strip. Certain dyes, such as eosin, safranin, iodine green and methyl green, followed a similar course.

Repeating these experiments with ferrous sulphate, some evidence was obtained of the same distribution, though in toxic concentrations. Many dyes gave clear evidence of their ready diffusion along the cellulose walls of the cortex and complete or partial stoppage at the endodermal surface. This distribution was clearly shown by cutting sections of roots of *Vicia Faba* after 2-24 hours in solutions

of dyes, particularly good results being obtained with Neutral red (0.1 per cent.), Malachite green (0.02 per cent.), and eosin (0.1 per cent.) and Hofman's blue in the presence of  $\text{KH}_2\text{PO}_4$ . The cases cited by De Lavison, and the numerous cases met with in the course of this work, make it clear that there are many water soluble substances which readily penetrate the cellulose walls of the piliferous and cortical cells along with the water in which they are dissolved, but which are completely blocked at the endodermal surface, or enter at a much reduced rate across the protoplasts (Pl. II, fig. 1).

That the processes of water entry and salt entry are distinct is further shown by the changes in concentration and composition of a culture solution which take place as the result of the growth of a plant. Hoagland(8) found that reduction in transpiration was not associated with anything like a proportionate decrease in the absorption of ions. As a general result he found also that Ca, Mg and  $\text{SO}_4$  were removed from the solution more slowly than water so that the concentration of these ions in the external solution increased, whilst the reverse was true of K and  $\text{NO}_3$ .

Plasmolysis in salt solutions is an indication of the difficulty with which salts will enter living protoplasts, and recovery from plasmolysis, as usually interpreted, is an indication that they may enter. Salts may then be regarded as entering the cells of the plant from aqueous solution by a process of diffusion, but the results of Hoagland(9, 10) and his colleagues in California on the entry of salts into the large internodal cells of *Nitella*, suggest that this is probably not a case of simple diffusion from one aqueous solution to another according to the diffusion gradient. It is possible on puncturing the large cells of *Nitella* to obtain the cell sap in sufficient quantity to compare the concentration of ions with that of the external solution. As a result it is found that the ions are frequently very much more concentrated in the sap than in the external solution, and this is so marked in some cases that certain ions, such as K, Cl and  $\text{NO}_3$ , may be almost completely removed from the external solution. For example, Cl may accumulate inside the cell until the concentration is as much as 100 times that of the same ion in the external solution(10). The possibility of an explanation on the grounds of adsorption or combination seems to be precluded by conductivity measurements on the extracted sap. Such a concentration of ions against the diffusion gradient can only take place as the result of the expenditure of energy, and Hoagland and Davis(10) find that

the accumulation of the ions is much more rapid if the cells are in the light. In the experiments with *Nitella* it was also found that ions escaped from the cells if the  $pH$  of the external medium became too low.

In the case of roots of higher plants, it is more difficult to obtain uncontaminated sap, but the comparison of expressed sap with the external solution shows the former to have a higher concentration. In this connection, especial interest attaches to the results of Trubetskova (23), who compared the concentration of the stelar sap as collected by bleeding, with that of the external solution. Thus similar relationships to those described by Hoagland for *Nitella* exist in the higher plants, the stelar solution being much more concentrated in respect of certain ions than the external solution. The relative concentration in the sap of one ion to another bears no relation to their relative concentration in the external solutions. The results certainly suggest that a process of unilateral diffusion is taking place from the soil solution into the stelar sap, comparable with the diffusion from the external solution into the *Nitella* sap, the salts of the external solution diffusing in as far as the outer tangential walls of the endodermis. Further inward diffusion along the walls is prevented by the Casparian strip, penetration of the endodermis being possible only if the ions in question can enter the protoplast.

As the soil solution travels in the cortex, it comes in contact with the protoplasts of the cortical cells and finally with those of the endodermal cells. If the living root cells behave like the *Nitella* cell, all these will accumulate ions. Hoagland points out, however, that this process of accumulation is relatively slow, and his results were obtained only after the exposure of the *Nitella* cells to the solution for a number of days. Consequently, we may assume that the soil solution penetrates as far as the endodermis, little altered by its passage across the cortex, and the endodermal cells themselves will likewise accumulate ions from the soil solution present in the outer tangential walls of the cells.

The endodermal cells are in the peculiar position of being bathed on the inner and outer faces by different solutions. Of these, the stelar solution is in contact with tissues which are growing and differentiating in the absence of air spaces, and one would anticipate that the accumulation of carbon dioxide would render this solution relatively acid. In view of Hoagland's result that an increase of the acidity of the external solution caused a loss of ions from the cell of *Nitella*, it may be predicted that ions may be lost from the



endodermal protoplasts where they are in contact with the relatively more acid stelar sap. The application of the facts for *Nitella* to the case of the endodermal cell opens the possibility of continuous entry of salts across the endodermis, ions being accumulated by the cell from the soil solution to the outside, and then being liberated on the inner side owing to the greater permeability of the cell on this side due to greater acidity. The results of Ursprung and Blum<sup>(24)</sup> are suggestive in relation to this view. By the use of their method of measuring suction pressure, they find that the endodermal cells have a high suction pressure on their outer surface and a low one on the inner surface, this being in good agreement with the conclusions based on application of Hoagland's experimental results to the problem of the entry of salts into the root.

Inside the vascular cylinder, the xylem and phloem groups alternate and this suggests the possibility of differential entry of salts opposite the relatively acid xylem and the relatively alkaline phloem. There are several facts which point to a difference between the tissues lying immediately outside the xylem and phloem, one of the most striking being the staining effects of dyes on sections of living roots. If roots of plants with relatively few xylem groups are sectioned and stained with dyes, the excess dye being washed out with water (or in some cases, preferably water with the addition of  $\text{KH}_2\text{PO}_4$  or  $\text{NaHCO}_3$ ), conspicuous patches of cells remain stained. These patches are always situated opposite to the xylem groups and extend from the cells immediately in contact with the xylem elements across the endodermis including a group of inner cortical cells (Pl. II, figs. 2 and 3). These staining effects associated with other phenomena, such as the origin of lateral roots opposite the xylem and the earlier development of the endodermis opposite the phloem, are probably all connected with the fact that the cells opposite the xylem groups are slower to differentiate than those opposite the phloem. There is no direct evidence as to what effect this difference between the tissues opposite xylem and phloem respectively in the absorbing zone may have upon the entry of salts into the stele.

#### SUMMARY

1. The absorbing region of the root is the area lying between the apical meristem and the regions with completely suberised membranes.
2. In the absorbing region the root cortex may be looked upon as a spongework of cellulose walls, containing the protoplasts in its

meshes. This spongework is interrupted at the endodermis by the Casparian strips. The superficial cells extend to form root hairs, when their external walls pass through the "amyloid" stage.

3. The entry of water must be considered in relation to soil moisture.

(a) When water is present in excess, and is free to move to the plant, the soil solution permeates the cellulose walls of the cortex and the cortical and endodermal cells take up water until they are turgid. The protoplasts of the endodermal cells act as a semipermeable membrane across which water is drawn from the outer tangential walls by the osmotic pull of the stelar solution in the inner tangential walls. Under these conditions the surface area of the root is not important.

(b) In drier soils water is less free to move in the soil, and under these conditions importance attaches to the increase in root surface due to growth and the production of root hairs. This importance is due in part to the increased contact between root and soil particles, and in part to the increase of root surface relative to endodermal surface, since the rate of entry of water from the soil into the root surface, necessary to maintain an adequate supply across the endodermis, diminishes in direct proportion to the ratio of root surface to endodermal surface. Therefore in comparatively dry soils root hairs perform an important function in relation to the entry of water.

4. Water and salt entry are quite independent. It is suggested that the cortical and endodermal cells accumulate ions from the soil solution in a manner similar to that shown by Hoagland to take place when a cell of *Nitella* is in contact with such a solution. These ions are liberated from the endodermal cells on the inner side, owing to the greater acidity of the stelar sap.

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## EXPLANATION OF PLATE II

- Fig. 1. T.S. Living root of *Vicia Faba* ( $\times 50$ ). The root was immersed in Aq. Neutral Red (0.1 %) for 2 hours before cutting. The section shows the dense staining of the outer cortex, less dense staining of the inner cortex, where the dye can be seen to be chiefly confined to the walls. The dye has penetrated groups of cells extending across the endodermis opposite the xylem groups. The dye rapidly diffuses.
- Fig. 2. T.S. Living root of *Vicia Faba* ( $\times 80$ ) 3 mm. from the apex. The sections were stained in Magdala Red, the excess being washed off with water containing  $\text{KH}_2\text{PO}_4$ . The section shows the staining of the protoplasts of the young cortical cells and of groups of cells extending across the endodermis into the stele in the region of the future xylem groups.
- Fig. 3. T.S. Living root of *Vicia Faba* ( $\times 80$ ) 14 mm. from apex. Sections stained as in Fig. 2. The section shows the staining of patches of cells extending from the inner cortex, and across the endodermis to the differentiating xylem elements.

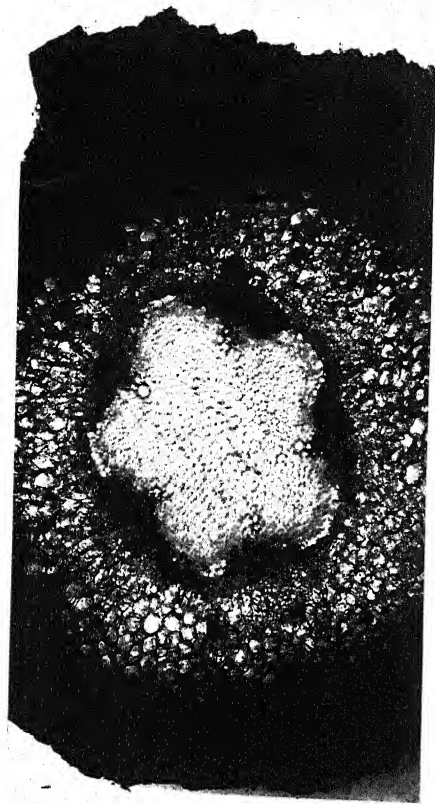


Fig. 1.

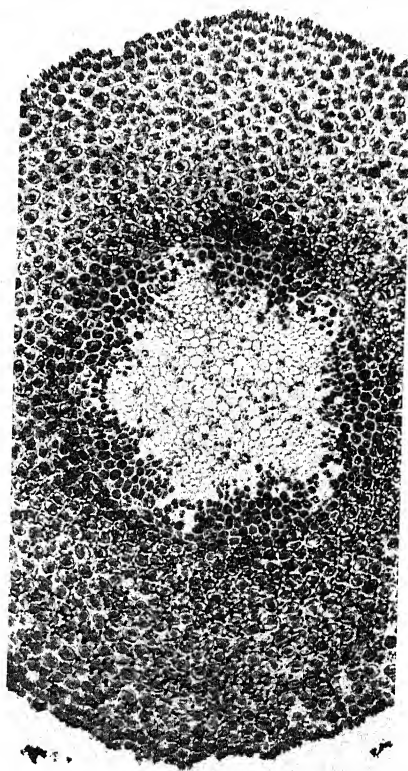


Fig. 2.

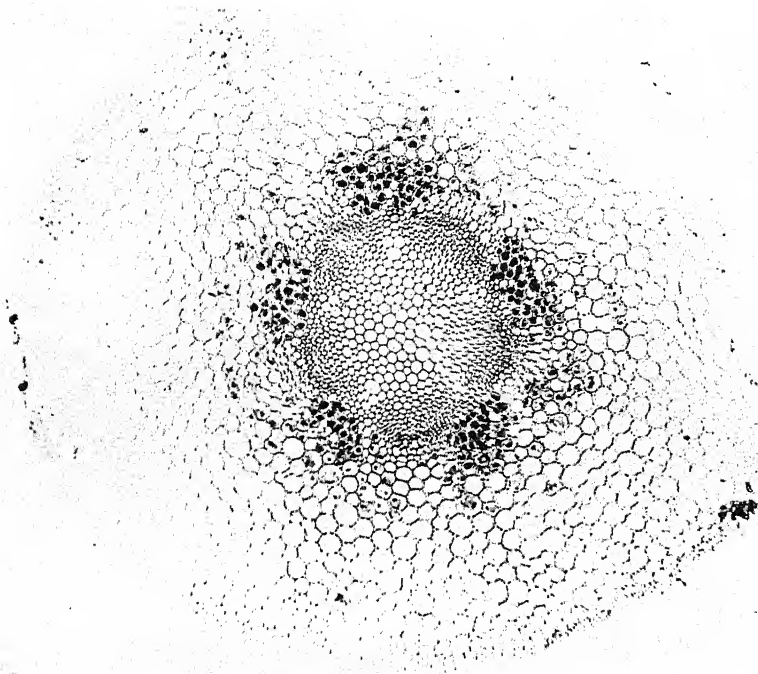


Fig. 3.



# THE ROOT AS AN ABSORBING ORGAN

## II. THE DELIMITATION OF THE ABSORBING ZONE

By LORNA I. SCOTT.

(With Plate III and 20 figures in the text.)

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### I. INTRODUCTION

FROM the point of view of functional activity, the root may be considered in three zones:

- (a) The meristematic zone.
- (b) The zone of extension.
- (c) The older parts of the root, where extension is no longer taking place.

The meristematic zone has already received considerable attention and it has been shown that this region of the root is relatively impermeable and consequently cannot play any part of importance in the process of absorption (15, 20).

The zone of extension behind the meristem is also the chief functioning zone in absorption. Its structure has been described in detail in an earlier paper (18) and discussed in special reference to the entry of water and salts. Behind the zone of extension, changes take place in the chemical composition of the walls of certain cells, especially of the exodermal and endodermal layers, and it is the object of the present paper to consider to what extent these later changes may delimit the functional absorbing region of the root. The various types of exodermis and endodermis have already been described, thanks chiefly to the Marburg School of botanists (6, 10), but it has been thought desirable to re-examine a few types with



special reference to development. The details have been worked out chiefly in the roots of *Funkia ovata*, but frequent comparison has been made with corresponding stages in a number of other plants, illustrating different types of root systems. The main features of the root systems considered may be summarised as follows:

(a) *Funkia ovata*

Branched roots. Endodermis and exodermis both tertiary, and with well-differentiated passage cells.

(b) *Hyacinthus orientalis*

Unbranched roots. Endodermis and exodermis tertiary with passage cells. The inner cellulose lamella of the endodermis and exodermis not so well developed as in *Funkia* and the passage cells less clearly defined.

(c) *Salix fragilis* ( $\times$  *pentandra*). *Water roots*

Much branched roots. Endodermis tertiary with passage cells. Exodermis tertiary without passage cells. In older stages cork is formed below the endodermis, and the cortex is exfoliated.

(d) *Rumex acetosella*

Branched roots. Endodermis tertiary and complete. Cortex exfoliated. The main roots are perennial and cork is formed under the endodermis. On the main roots are borne successive crops of transitory roots, which break through in the position of a former crop or from the bases of buds. These transitory roots die off without forming cork.

(e) *Vicia Faba*

Roots branched. Endodermis tertiary with passage cells. No exodermis and the cortex is retained practically throughout life, but becomes much torn by branch roots. Cork is formed under a tertiary endodermis in *Phaseolus vulgaris* and *Lupinus* sp., in which also the cortex is exfoliated.

The maintenance of the zone of extension and maximum absorption is dependent upon growth, whilst subsequent wall changes are not, so it is obvious that the distance from the root apex at which the later stages in differentiation of the endodermis and exodermis appear will vary with the rate of growth. In extreme cases, the absorbing region may be practically eliminated by the differentiation of these layers to within a very short distance from the apical



meristem, and sometimes this elimination may be made more complete by the impregnation of the walls of the cells of the piliferous layer and root cap with fatty substances also.

Obviously, seasonal variations of this kind are of importance in any consideration of the delimitation of the functional absorbing region of the root.

## 2. THE ENDODERMIS. GENERAL CONSIDERATIONS

In 1922, Priestley and North (12) published the results of a re-examination of the structure of the endodermis and they conclude that in development, the tangential walls undergo normal differentiation into a pectic middle lamella and cellulose lamella, whilst the radial and transverse walls become impregnated with lignin-like substances and derivatives of fatty acids at a very early stage, when they are still similar in nature to those of the meristem cells. The following measurements from a *Funkia* root show that the lignification of the radial walls may become recognisable at a very short distance behind the meristem and at about the same level as the differentiation of the first vascular elements.

Recognition of	Distance from root apex (including root cap)	Distance from apex of meristem (excluding root cap)
	mm.	mm.
1st phloem element	1.01	0.65
1st lignified Casparian strips on endodermal walls	1.22	0.86
1st lignified xylem	1.34	0.98

The close association of the development of the Casparian strip with the differentiation of the vascular tissues, and especially its earlier development opposite the phloem, led Priestley and North to suggest that the impregnating substances of the strip were probably liberated from the phloem and that these became fixed in the radial walls of the endodermal cells on coming in contact with the inwardly diffusing air from the cortex. The phloem obviously does not retain fat in its walls, as is shown by the ease with which these give the cellulose or even amyloid reactions (25). In certain Compositae, where especially large quantities of fatty substances are present, Tetley (19) has described the development of intercellular canals which appear immediately outside the endodermis opposite the phloem groups, and the evidence points to fatty substances of the nature of unsaturated

drying oils as the canal contents in the early stages. Not only is the first appearance of the endodermis connected in this way with the phloem, but as the endodermal cells pass into the secondary and tertiary stages, these also become evident first opposite the phloem, and the same fact applies to the development of pericyclic cork. As regards the substances which become fixed in the radial walls of the primary endodermal cells as the Casparian strip, the impregnation with lignin-like substances is generally accepted on the grounds of the usual microchemical reactions for lignin with phloroglucin and hydrochloric acid, or in Maule's reaction, and the removal of the substances responsible for these lignin staining reactions with oxidising reagents such as Eau de Javelle or chromic acid.

Evidence for impregnation with fatty substances is less easy to obtain by staining reactions and has not been so generally recognised in the literature. The Marburg School regard the Casparian strip as lignified only and Ziegenspeck<sup>(25)</sup> uses this fact in criticising de Lavison's results on the entry of dyes and salts into roots<sup>(7)</sup>. Priestley and North based the conception of fatty impregnation chiefly on the resistance to sulphuric acid, the relative impermeability, the effect of ethylene upon the formation of the strip during development, and the theoretical consideration that if unsaturated fatty acids are liberated on differentiation they would become fixed on coming in contact with air. More recently, Priestley and Rhodes<sup>(14)</sup> have shown that on macrochemical analysis the Casparian strip in the primary endodermis of *Hyacinthus orientalis* yields free fat and other fatty derivatives in some form of combination, yielding on saponification normal and oxidised fatty acids. This conception is also supported by van Wisselingh<sup>(23)</sup> who has succeeded in getting more direct microchemical evidence. He finds that in certain plants, as for example *Taraxacum officinale*, *Valeriana officinalis*, *Acorus Calamus*, etc., he can obtain typical droplets of a yellow fatty substance from the Casparian strip by the cerin acid reaction. The reaction is obscured in some plants by the contraction of the protoplasm into granules which adhere to the wall, but where this does not occur the reaction is sufficiently conclusive to bring van Wisselingh into complete agreement with Priestley and North.

The endodermis remains in the primary stage in the zone of elongation and apparently any cellulose deposited by the protoplast at this stage is counteracted by the stretching of the cell, so that the wall shows no appreciable increase in thickness. As soon as the cell ceases to stretch, however, any material deposited adds to the

thickness of the wall in the form of secondary lamellae. In most plants a suberin lamella is first laid down over the inner face of the primary wall and this stage—the secondary stage of Kroemer—is a common feature in Leptosporangiate ferns<sup>(13)</sup>, but is extremely rare in Angiosperms, where, with few, if any, exceptions, the endodermis immediately passes on to the tertiary stage and develops a cellulose lamella internal to the suberin lamella.

Priestley and North<sup>(12)</sup> regard the suberin lamella as laid down in a basal wall substance, which is probably "akin to the inner carbohydrate lamellae of the tertiary stage." Van Wisselingh<sup>(23)</sup> criticises this view on the grounds of the readiness with which the tertiary lamella separates from the primary wall and the ease with which the suberin boils out into droplets on saponification with potash and in the cerin acid reaction. These facts he regards as indicating that the suberin is not laid down in a basal wall substance.

On this point it is difficult to obtain direct evidence, but comparison of the behaviour of the suberin lamella of the Angiosperm with that of the Fern<sup>(13)</sup> certainly favours the view that a basal substance is present in the suberin lamella of the Angiosperm, whilst it is absent in the Fern. In the Fern the suberin lamella develops by the coalescence of droplets of oily consistency, which subsequently set to a hard varnish-like layer, which in young cells readily breaks up into droplets again on warming. In older parts of the plant the suberin lamella is frequently found to have cracked. No tertiary cellulose lamella is developed internal to it. In the Angiosperm, no stage has been observed where the suberin lamella is represented by free droplets and similarly in older stages there does not seem to be any tendency for the suberin layer to crack. This is especially striking when one considers the strain that must often be put upon the endodermis by the increased volume of the stele due to cambial activity. In the case of willow roots, measurements of the tangential diameter of the suberin lamella showed an increase of about 40 per cent. as a result of this strain, yet there was no indication of discontinuity of the suberin. Though it is improbable that there should be complete cessation in the deposition of cellulose by the protoplast between the laying down of the primary and tertiary lamellae, it is possible that these two layers may differ to some extent in their properties, owing to the fact that the primary wall is laid down chiefly during the period of root extension and the tertiary lamella after this has stopped. The experiments to be described suggest a possible difference in the swelling properties; but chiefly the difference

in behaviour of the primary and tertiary layers would appear to be due to the close association of the primary wall with the lignified and suberised Casparian strip and the suberin lamella.

*Experiment I.* Steles of *Funkia* were drawn from the roots and either boiled directly in potash, when strips of endodermis could be easily separated from the other tissues on transferring to water, or the strips were removed from fresh steles with a knife and subsequently treated with potash.

Strips of fresh endodermis show the refractive, undulating Casparian strip running round each cell. The pitted cellulose of the primary tangential wall is seen in surface view of the passage cells,

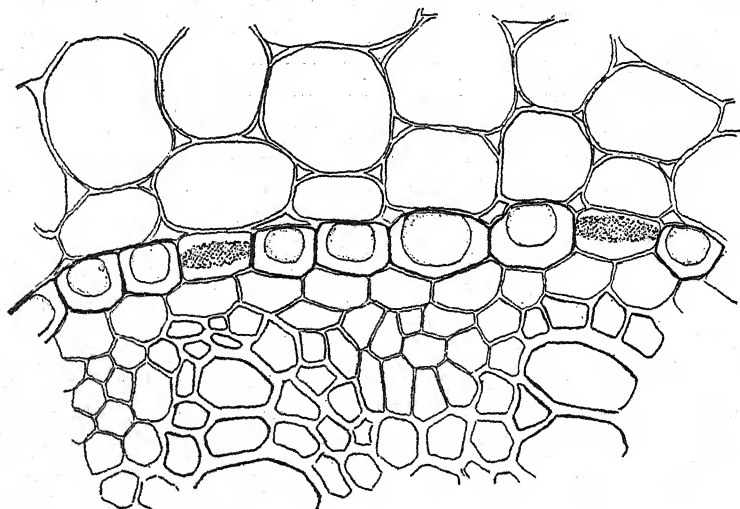


Fig. 1. T.S. Root of *Funkia ovata* ( $\times 400$ ) showing the tertiary endodermis with passage cells opposite the xylem groups. The tertiary cellulose lamella is scarcely developed over the outer tangential wall. The protoplasts of the passage cells are contracted but remain attached to the Casparian strip.

but is obscured by the tertiary cellulose lamella of the remaining cells, except where this is incomplete over the outer tangential wall (Figs. 1 and 2).

On gentle warming in dilute potash the cells undergo a slight expansion amounting to about 2-3 per cent. on the length of the cells, but the most obvious change is a swelling of 100-150 per cent. in the thickness of the tertiary lamella, which consequently almost blocks up the cell lumen.

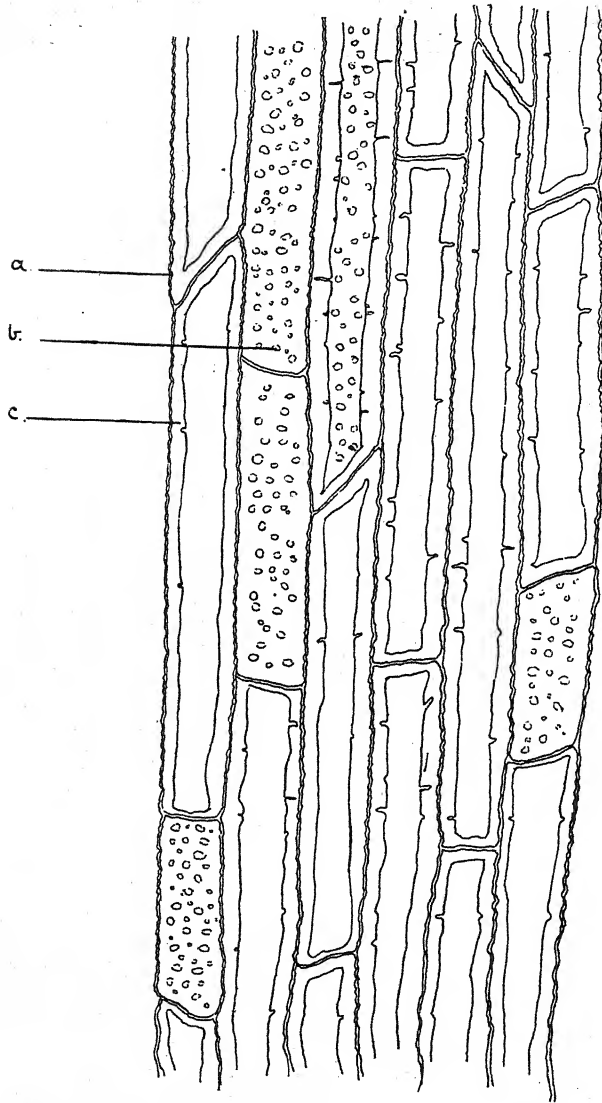


Fig. 2. *Funkia ovata*. Surface view of the endodermis from a fresh root ( $\times 350$ ), viewed from the cortical side. *a*, undulating Casparian strip; *b*, passage cell showing pitted cellulose of tangential wall; *c*, tertiary cellulose lamella.

On boiling in concentrated potash the whole cell undergoes contraction in length and width up to about 11 per cent. (on length) and this is accompanied by more pronounced undulation of the

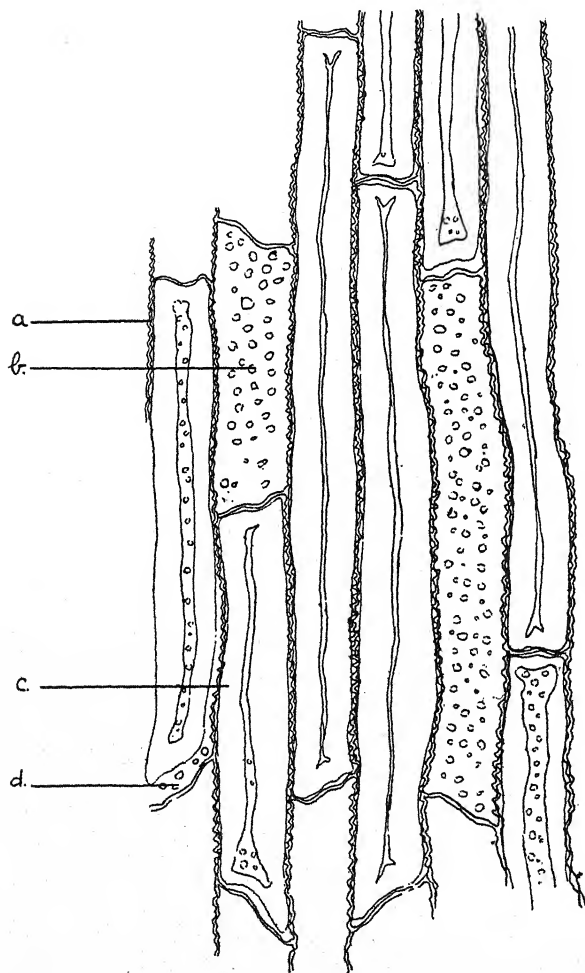


Fig. 3. *Funkia ovata*. Endodermal strip after boiling in strong potash ( $\times 350$ ). *a*, undulating Casparian strip; *b*, tangential wall of passage cell; *c*, tertiary cellulose lamella; *d*, primary wall exposed by contraction of *c*.

Casparian strip. The thickness of the tertiary lamella does not show any further measurable change but this lamella tends to contract slightly more than the primary wall in length as shown by separation

of these layers at the ends of the cells, by which process the cellulose of the primary wall is exposed (*d*, Fig. 3). From the more pronounced undulation of the lignified Casparian strip, it is obvious that the tendency of this layer as regards contraction differs from that of the cellulose of the primary wall and the separation of the tertiary

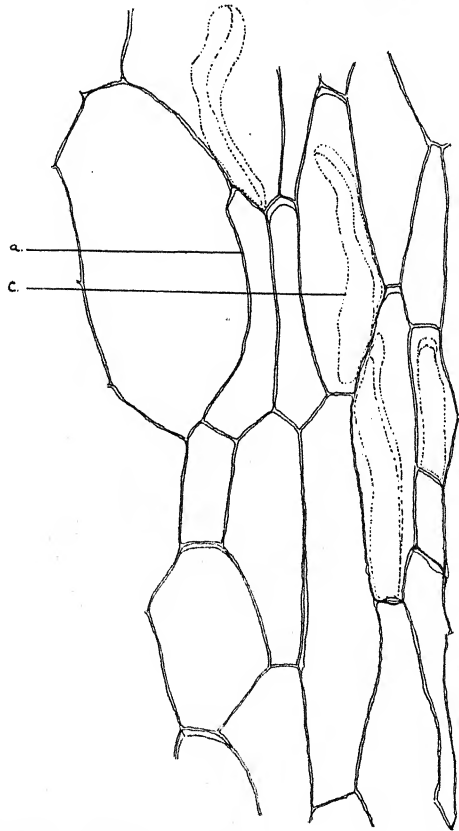


Fig. 4. *Funkia ovata*. Endodermal strip after boiling in strong potash and treating with zinc chloride in hydrochloric acid ( $\times 125$ ), showing (*a*) the straightened Casparian strip, and (*c*) the outermost layer of the tertiary cellulose lamella.

lamella might be associated with strains set up in the wall in this way, or with a weakening of the wall at the line of separation by saponification of the suberin lamella. The separation is no proof of the presence or absence of a basal substance to the suberin lamella.

1. Some of the strips boiled in potash were treated for 48 hours

in Schweitzer's solution. The outer part of the tertiary lamella remained undissolved.

2. Other strips were treated with zinc chloride in hydrochloric acid. The Casparian strip straightened out, probably on removal of the cellulose of the primary wall. The tertiary lamella dissolved except for a thin outermost layer (Fig. 4). A possible explanation of

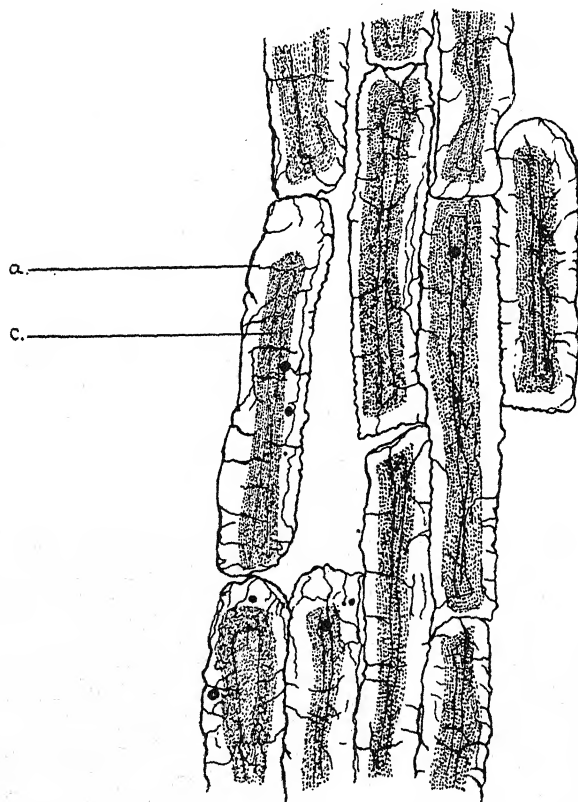


Fig. 5. *Funkia ovata*. Endodermal strip ( $\times 350$ ) after boiling in Schultze's macerating fluid and staining with Sudan III for fats and iodine and zinc chloride for cellulose. *a*, basis of Casparian strip and suberin lamella (stained red with Sudan III); *c*, tertiary cellulose lamella.

these results would be, that on removal of the suberin a basal substance akin to cellulose remains and this contracts away from the primary wall with the tertiary lamella; this is more resistant to cellulose solvents and is left when the purer cellulose of the tertiary lamella dissolves in zinc chloride in hydrochloric acid.



*Experiment II.* As in the previous experiment, steles were drawn from *Funkia* roots and treated either whole or in the form of endodermal strips removed from fresh roots. The treatment in this case consisted in boiling for a few seconds in Schultze's macerating fluid. The reagent attacks the lignified Casparian strip, with the result that the cells easily become separated from one another. Some of the suberin of the secondary lamella also boils out into globules. On staining this material with Sudan III and subsequently with iodine and zinc chloride, preparations were obtained showing isolated endodermal cells, with a much and irregularly wrinkled outer membrane stained red with Sudan III surrounding the much contracted tertiary cellulose lamella stained blue (Fig. 5). The outer cell membrane showed contraction compared with fresh endodermal cells, the degree of contraction varying with the duration of boiling. With short treatments this amounted to about 3 per cent. (on length). In this process again the tendency is for the cellulose to contract. The tertiary lamella does contract considerably and breaks away from the primary wall, which is prevented from more than a limited contraction by the incomplete removal of the suberin lamella, which does not tend to contract. If the suberin is removed by boiling these cells in potash, the primary wall, which now stains like cellulose, undergoes a further contraction of about 10 per cent. in length. Fig. 6 shows that the primary wall is now contracted almost as much as the tertiary lamella in length, but still remains wrinkled and inflated to some extent.

1. Treatment with zinc chloride in hydrochloric acid after Schultze's fluid removes the cellulose from the primary wall, and the Casparian strip and suberised layer straighten out to two or three times the original size of the cell. The tertiary cellulose appears to be protected from the reagent.

2. After Schultze's fluid followed by potash, zinc chloride in hydrochloric acid dissolves the tertiary lamella also. The part of the outer membrane remaining may represent in part the basis of the suberin lamella.

In these two experiments, the endodermal wall has been attacked from different points. In Experiment I the suberin is attacked, and if a basis is present similar to cellulose the probability is that rupture of the wall will take place between the primary wall and the secondary lamella. In Experiment II, the strong oxidising reagent removes the lignin, but does not remove more than a small proportion of the suberin, with the result that rupture will probably take place

between the secondary and tertiary lamellae. The evidence is obviously inconclusive for the presence of a basal substance to the suberin lamella, but these reactions do indicate that the separation of the tertiary lamella from the primary wall can still less be taken as conclusive evidence for its absence when the treatments involve strong volume changes in a wall of complex nature.

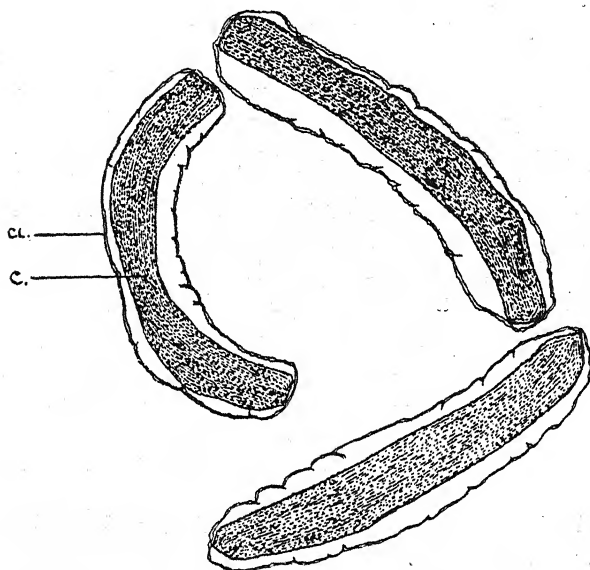


Fig. 6. *Funkia ovata*. Endodermal cells ( $\times 350$ ) after (1) boiling in Schultze's macerating fluid, (2) heating in potash, and (3) staining with iodine and zinc chloride. *a*, basis of Casparian strip and primary wall; *c*, tertiary lamella.

### 3. THE ENDODERMIS. DISTRIBUTION AND FUNCTION

In the majority of Angiosperms the primary endodermis is a transient stage and at cessation of growth some or all of the cells usually develop a secondary suberin lamella and an inner cellulose lamella, so passing into the tertiary stage. As already stated, it is a general fact that these later stages are first initiated opposite the phloem groups, and in Monocotyledons, where there is no secondary growth, it is a common feature for only those cells opposite the phloem to pass into the tertiary stage, whilst those opposite the xylem remain permanently in the primary stage as passage cells (Fig. 1), e.g. *Funkia ovata*, *Hyacinthus orientalis*. In Dicotyledons,

although the cells opposite the xylem remain longer in the primary stage, a complete tertiary endodermis is usually developed eventually. This happens relatively early in the fine rootlets of *Rumex acetosella*,

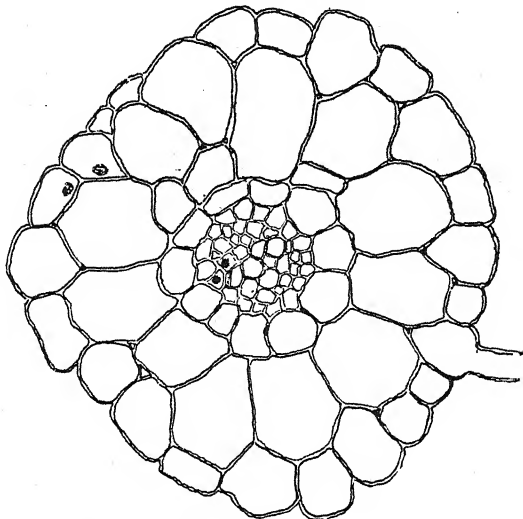


Fig. 7. T.S. *Rumex acetosella* root ( $\times 480$ ). Primary endodermis and living cortex.

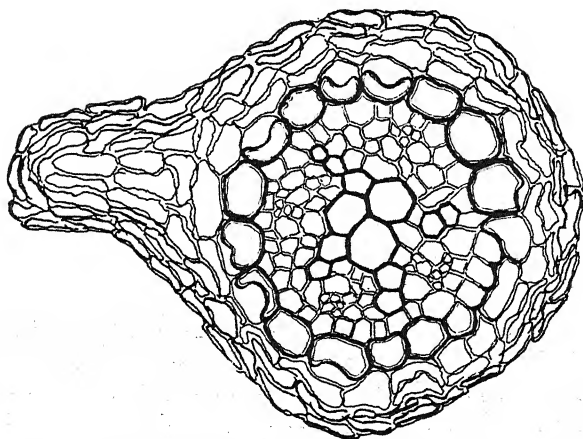


Fig. 8. T.S. *Rumex acetosella* root ( $\times 480$ ) showing the collapsed cortex outside the tertiary endodermis.

but later and probably not until the ring of phloem has been completed by secondary growth in many larger roots, e.g. *Salix fragilis* (water roots), *Phaseolus vulgaris*, *Lupinus* sp.

The laying down of a suberin lamella round each endodermal cell appears to prevent or at least much reduce the exchange of substances across this layer. The most significant evidence for this is the collapse and exfoliation of the cortex outside a complete tertiary endodermis, e.g. *Rumex acetosella* (Figs. 7 and 8), and in larger roots the earlier death of that part of the cortex lying outside parts of the endodermis where the cells have developed a suberin lamella, e.g. *Salix fragilis* (Fig. 9).

Further evidence is afforded by the fact that in Dicotyledons with a complete secondary or tertiary endodermis, the substances

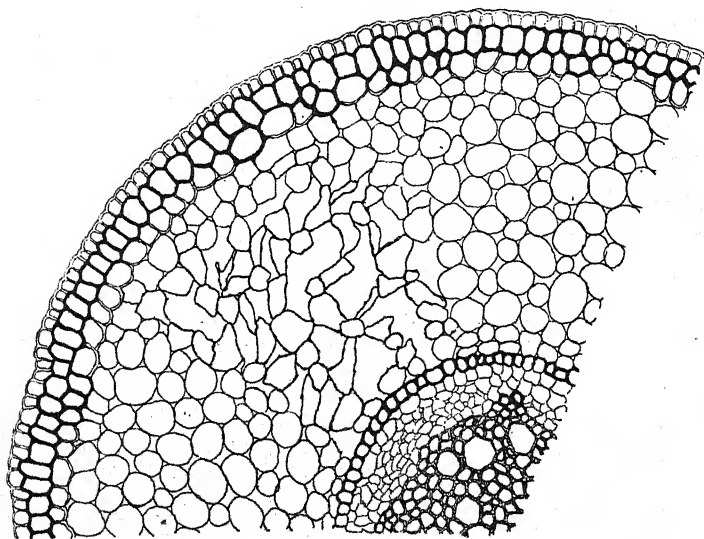


Fig. 9. T.S. Water root of *Salix fragilis* ( $\times$  *pentandra*) ( $\times$  45). Section 5.4 cm. from root apex at end of May 1926, showing the disintegration of the cortex outside the suberised part of the endodermis opposite the phloem. A suberised exodermis is present.

which lead to the development of a cork phellogen, are apparently retained inside the endodermis and the cork is pericyclic in origin, e.g. *Salix fragilis*, *Lupinus* sp., *Phaseolus vulgaris*, *Rumex acetosella* (main roots). A limited number of Dicotyledonous roots are recorded as having a cortical phellogen, but in such cases as have been re-examined, it has been shown that the endodermis is not in the complete secondary or tertiary condition. The Compositae show considerable variation in this respect and it was shown by Tetley (19) that exogenous cork occurred in species with either a primary

endodermis or a secondary endodermis which allowed of leakage across it in the region of the secretory canals. The suberin lamella of Compositae appears to be less rigid than that of most Dicotyledons and is more easily ruptured when strained by cambial growth. Some species of *Solanum* furnish a similar explanation for exogenous cork, for in *S. dulcamara* var. and *S. capsicastrum* var., the endodermis is at least partly secondary, but this becomes squashed and ruptured by secondary increase in the vascular tissues (based on unpublished work of Miss L. M. Woffenden). In the aerial root of *Philodendron erubescens*, the secondary endodermis is incomplete and in *Monstera deliciosa* Liebm. it remains in the primary stage (16). These facts taken in conjunction with experimental results on the entry of dyes and salts (7) confirm the view that a complete secondary or tertiary endodermis practically prevents any exchange between stele and cortex and consequently puts a limit to the absorbing region of the root.

In many Dicotyledons, cambial activity continues inside the secondary or tertiary endodermis, and this must put considerable strain on the endodermal layer, which in order to accommodate the increase must either rupture as in the *Solanum* spp. described above, or be able to undergo considerable extension. In plants where deposition of cellulose inside the endodermal cell is long continued, the cells are capable of considerable extension and it is chiefly in connection with this that the question of the relation of the basal substance to the suberin lamella is one of some importance. The fact that the suberin lamella can undergo such extension without rupturing seems strong evidence for the presence of such a basal substance.

In a stretched tertiary endodermis of this kind it is a common feature for the cells to become chambered by the development of cellulose partitions, which run in radial and transverse directions (Fig. 10). In the present work, *Salix fragilis* showed considerable development of such partitions and it was also evident to some extent in *Vicia Faba* in addition to the numerous cases cited in the literature, e.g. *Gentiana lutea*, *Taraxacum officinale* (23), other Compositae (19), *Ricinus communis*, *Helleborus niger*, etc. (6).

#### 4. DEVELOPMENT AND STRUCTURE OF THE EXODERMIS

##### *Funkia ovata*

In the differentiation of the root tissues, vacuolation starts in the middle region of the cortex and proceeds inwards and outwards from this point, with the result that the superficial or future piliferous

layer and the hypodermal or future exodermal layer are relatively late in differentiating. These differences in the time of vacuolation cause the air spaces, which are best developed in the middle cortex, to become progressively smaller towards the periphery of the root where ultimately the piliferous and hypodermal layers fit on to one another without air spaces. In the meristematic region, the hypodermal cells all appear equivalent, but the last division results in

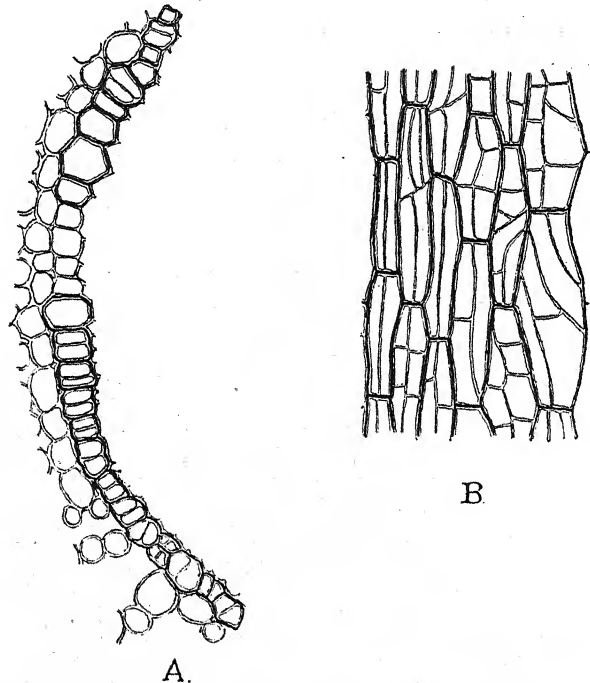


Fig. 10. Water roots of *Salix fragilis* ( $\times$  *pentandra*) showing chambering of the tertiary endodermal cells by cellulose partitions. Sections cut in March in a region at the upper end of the previous season's growth. A ( $\times$  285). T.S. showing passage cells and suberised cells. B ( $\times$  225). Surface view.

cells of very unequal proportions (Fig. 11), long cells, which are usually six-sided in surface view and short-cells which are usually four-sided. The cells of the two types alternate in the vertical direction, the regularity being interrupted only occasionally by the juxtaposition of several long cells. For a distance varying with the rate of growth, the walls of long and short cells give cellulose reactions, but with increasing age a stage is reached when a thin and

apparently central region of the radial walls gives reactions for fat (Sudan III), and lignin (phloroglucin and hydrochloric acid and Maule's reaction). This staining region is most marked at the end of the wall nearest to the cortex (Fig. 12). From this stage, the further differentiation of the long and short cells is not the same and that of the long cells will be considered first.

In the long cells the suberisation soon extends to the tangential walls and a complete suberin lamella is developed all round the cell quite similar to the secondary suberin lamella of the endodermal cells. Lignification also extends to the other walls, but the intensity of the reactions in this case is greatest in the outer tangential walls and gradually falls off in a radial and inner tangential direction.

In addition to the suberin lamella, an inner cellulose lamella is deposited as in the case of the tertiary endodermal cells.

Occasional short cells may develop a suberin lamella and inner cellulose lamella like the long cells, and Francke(3) noticed similar behaviour of some short cells in *Asclepiadaceae*. The majority of the short cells fail to develop a suberin lamella and also differ from the long cells in the fact that the tertiary cellulose lamella is scarcely developed on the inner tangential and radial walls, but is very thick and slightly lignified on the outer wall (Figs. 13 and 14). These thick cellulose "caps" are developed relatively early according to van Wisselingh and Kroemer(6), and coincidentally with the development of the suberin lamella in the neighbouring long cells(6). The walls of the superficial root cells are also impregnated to a slight extent with fatty and lignin-like substances, but this is variable and never confined to a definite region of the wall as in the case of the suberin lamella of the

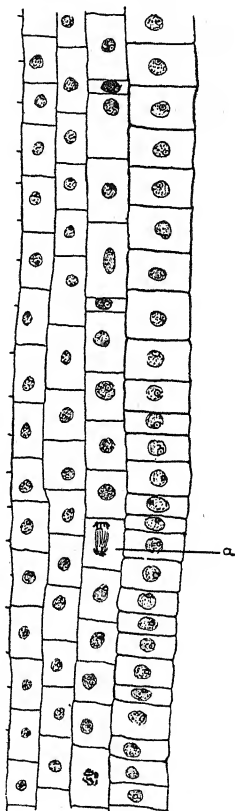


Fig. 11. *L.S. Funkia ovata* root. Cell *a* is about the 83rd from the apex of the cone of meristem. The lower half of the figure shows the radial extension of the piliferous cells and a stage where the hypodermal cells are all alike. The upper half shows the differentiation of the hypodermis into long and short cells.

long cells of the exodermis or of the endodermis. However, the impregnation of the walls of the superficial and hypodermal layers which are in contact, is sufficient to cause these two layers to hold together under most macerating treatments. For example, after (a) Eau de Javelle, or 1 per cent. ammonium oxalate after prolonged treatment with 25 per cent. alcoholic hydrochloric acid, (b) Schultze's macerating fluid in a warm oven for 24 hours, or

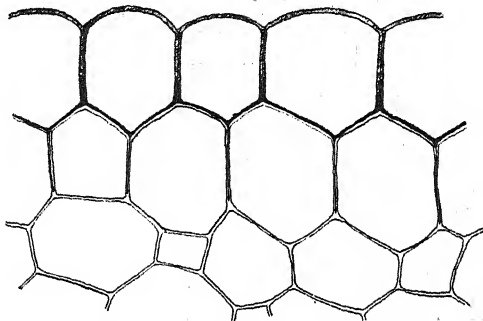


Fig. 12. T.S. *Funkia ovata* root ( $\times 485$ ). Section stained in Cotton red showing impregnation of the walls of the superficial cells, and of a middle region of the radial walls of the exodermal cells.

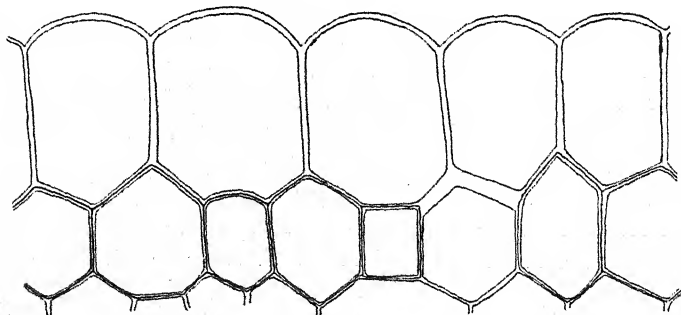


Fig. 13. T.S. *Funkia ovata* root ( $\times 412$ ) showing suberised exodermal cells and a passage cell in transverse section.

(c) concentrated chromic acid, the parenchymatous tissues break down, but the piliferous and exodermal layers remain intact and attached to one another. More vigorous treatment such as (a) boiling in Schultze's macerating fluid, or (b) concentrated chromic acid followed by rapid treatment with Eau de Javelle, breaks down the middle lamella of these layers also, and the cells separate. The isolated cells obtained in either of these two ways can be stained



with Sudan III, showing that each cell has a distinct suberin lamella, but the fact that the exodermis only macerates after treatments which remove some fat as well as lignin, suggests that the fatty impregnation does extend to some extent to the middle lamella.

If the isolated cells obtained by boiling in Schultze's macerating fluid are stained with Sudan III and then with iodine and zinc chloride, the long cells and some of the short cells appear very similar to the tertiary endodermal cells treated in the same way. The outermost layer is wrinkled and stained red, whilst the inner cellulose lamella is contracted away from it. Apparently, the tendency is for the cellulose to contract and the suberin layer to expand, as in zinc chloride and hydrochloric acid the cellulose is removed and the fat-staining layer expands to about three times the original size of the cell (Fig. 15). Boiling in Schultze's fluid removes the lignification and suberisation from the superficial cells and the walls then give cellulose reactions.

Boiling in strong potash causes contraction of the cells, and usually the tertiary cellulose lamella contracts more than the primary wall and separates from it.

The suberin lamella and inner cellulose lamella of the long exodermal cells behave in a similar manner to reagents as the corresponding layers of tertiary endodermal cells, but differences exist between the primary walls in the two cases. In the exodermis there is nothing quite comparable with the very resistant Casparian strip of the endodermal cell with its embedded protoplast. The difference probably lies in the state of differentiation at the time impregnation takes place, as in the exodermis the walls at this time already consist of middle lamella and cellulose lamella, whilst those of the endodermis are only just emerging from the meristematic condition.

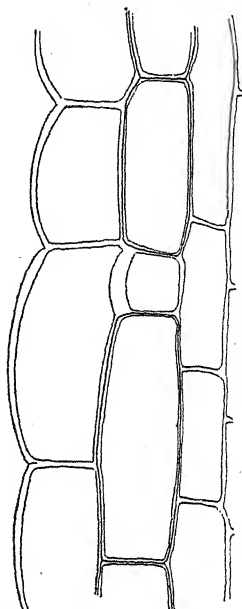


Fig. 14. L.S. *Funkia ovata*; part of section of root ( $\times 360$ ) showing suberised exodermal cells and a passage cell in longitudinal section.

#### *Hyacinthus orientalis*

The exodermis in *Hyacinthus* consists of long suberised and shorter unsuberised cells, but the difference between the two types

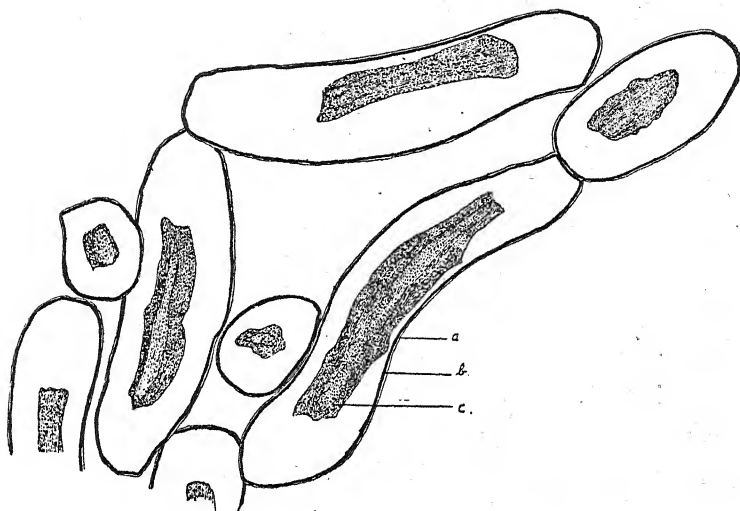


Fig. 15. *Funkia ovata*. Exodermal cells ( $\times 260$ ) after (1) boiling in Schultze's macerating fluid, (2) staining in Sudan III, (3) iodine and zinc chloride in hydrochloric acid. *a*, basis of primary wall; *b*, suberin lamella; *c*, tertiary cellulose lamella.

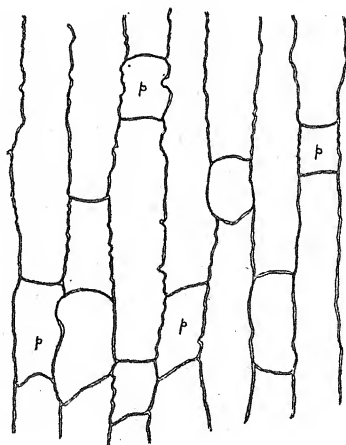


Fig. 16. *Hyacinthus orientalis*. Exodermal strip ( $\times 150$ ). The cells marked *p* are unsuberised passage cells. The remaining short cells and the long cells are suberised.

of cell is not nearly so striking as in the case of *Funkia* (Fig. 16). The suberisation and lignification appear first on the radial walls and this stage persists for a considerable time, before the suberin lamella is completed round each long cell (Fig. 17). A thin tertiary cellulose lamella is developed inside the suberin lamella in both endodermal and exodermal cells, but in the unsuberised exodermal cells the thick "caps" are not developed, the cellulose lamella being thicker on the inner tangential wall than on the outer. Apart from

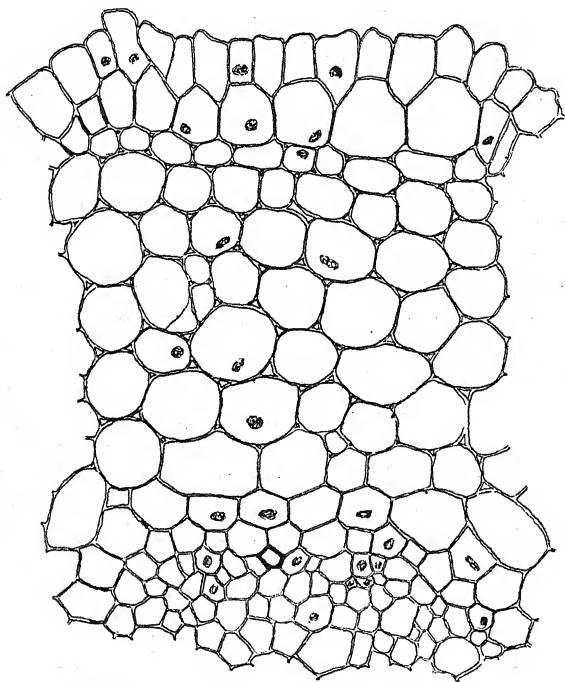


Fig. 17. T.S. *Hyacinthus orientalis* root ( $\times 200$ ). Section at about 3 mm. from the root apex, showing a primary endodermis and an early stage in the suberisation of the exodermis with only the radial walls suberised.

slight differences of this kind, the *Hyacinthus* exodermis is of the same general type as that of *Funkia*, but the differentiation into long and short cells is so slight that it is not easy to distinguish the two types without the evidence of fat stains or the behaviour of the cells in absorption. *Hyacinthus* is described as having a uniform exodermis consisting of suberised cells only by Nicolai, but Kroemer<sup>(6)</sup> points out that the types of this author need verification as he did not demonstrate suberisation.

*Salix fragilis* ( $\times$  *pentandra*). *Water roots*

The exodermis is of the uniform type, consisting of suberised cells only, which are similar to those of *Hyacinthus*.

The suberisation in this case is not strictly confined to the single hypodermal layer, but affects in a somewhat irregular way the cells of the next inner few cortical layers, which differentiate like typical suberised exodermal cells (Fig. 9). Eventually the cortex with the exodermis is exfoliated as a result of the completion of the secondary endodermis and the development of pericyclic cork.

*Vicia Faba*

In this type the endodermal passage cells appear to be maintained and the cortex is consequently not exfoliated, but in spite of this no typical exodermis is developed. It is possible that this may be attributed to a scarcity of fat in the plant as the cortical tissues give cellulose reactions readily with iodine and zinc chloride without any previous treatment with potash or Eau de Javelle as is so commonly necessary. In older parts of the root, the outermost layer of cells gives a brown colour with iodine and zinc chloride and appears to be relatively impermeable in absorption experiments, but it does not give any fat reaction with Sudan III.

Numerous branch roots are developed and the secondary endodermis of these unites with that of the main root. The enlargement of these branch roots causes considerable rupturing of the cortex. Cork is not formed in *Vicia Faba*, but typical pericyclic cork occurs in *Phaseolus vulgaris* and *Lupinus* sp. with exfoliation of the cortex. If *Vicia Faba* roots were preserved in alcohol a blackening of the tissues made it difficult to see subsequent colour reactions. This darkening of the tissues was prevented by bubbling sulphur dioxide (prepared by adding 80 per cent. sulphuric acid to sodium sulphite) into the alcohol at the time of preservation (26).

#### 5. MACROCHEMICAL ANALYSIS OF THE EXODERMIS OF *HYACINTHUS*

Material for analysis was obtained by allowing roots to rot in water, after which the exodermis was separated by hand. The material was well washed in water and finally dried. In this way 6.12 gm. of dry matter were obtained for investigation. The analysis was carried out by Dr E. Rhodes and the details of the method are the same as those used in the analysis of cork (17) and endodermis (14). The outlines of the method employed are as follow:

Dry weight of material

Continuous extraction with chloroform in a Soxhlet apparatus

Chloroform extract  
On evaporating off  
chloroform  
Weight of chloroform  
soluble matter

Residue  
Saponify with alcoholic soda and  
filter off alcoholic liquid. Boil residue  
with absolute alcohol. Distil the  
combined alcoholic extracts to dry-  
ness and warm the residue in distilled  
water. Thus an aqueous solution and  
suspension are obtained. Shake with  
ether and separate

Ether extract.  
Evaporate off the ether.  
Wt. of unsaponifiable  
material

Aqueous solution.  
Acidify with dilute hy-  
drochloric acid. Shake  
the suspension of free  
acids with petrol ether  
and separate

Petrol ether extract.  
Evaporate off petrol  
ether.  
Wt. of normal acids

Aqueous suspension.  
Filter through  
weighed filter paper.  
Wt. of oxy-acids

Results

Wt. of dry exodermal tissue	6.12 grm.
Wt. of chloroform soluble matter	0.245 grm. (4 % on dry wt. of tissue).
Wt. of unsaponifiable material	0.419 grm. (6.8 % on dry wt. of tissue).
Wt. of normal acids	0.1844 grm.
Wt. of oxy-acids	0.2236 grm.

$$\text{Ratio} \frac{\text{Wt. of normal acids} + \text{wt. of oxy-acids}}{\text{Wt. of chloroform soluble material}} = 1.66.$$

$$\text{Iodine number of chloroform soluble fat} = 32.3.$$

The oxy-acids contain traces of phellonic acid as indicated by Gilson's test, a violet colour being produced when, to material moistened with alcoholic iodine, sulphuric acid is added (4). Phellonic acid was identified by Gilson (4) and van Wisselingh (21) from the cork of most plants examined, but according to van Wisselingh (22) it is not present in cuticle. No definite evidence for its presence was found in investigation on endodermis (12, 14) or potato cork (17). The ratio  $\frac{\text{Normal acids} + \text{Oxy-acids}}{\text{Chloroform soluble fat}}$  of 1.66 is intermediate between the values for potato cork which are about 3.3 to 3.6, and those for tertiary endodermis which range from 1.08 in fresh material to 1.59 in stored material of *Potamogeton*.

## 6. EFFECT OF THE EXODERMIS ON ABSORPTION

In the case of the endodermis, evidence has been put forward to show that the development of a suberin lamella renders the cell relatively impermeable and this conclusion would be expected to hold for the cells of the exodermis also, where a similar suberin lamella is developed. Consequently an exodermis of the uniform type as found in the water roots of *Salix*, in which all the exodermal cells develop a suberin lamella, would be practically a complete barrier against continued root absorption, but the presence of short, unsuberised cells in the exodermis of *Funkia* and *Hyacinthus* suggests the possibility of a continued, though restricted entry of water and solutes. The following experiments were carried out to test this conclusion.

Roots of *Funkia* plants which had been growing in water were transferred to 0.5 per cent. lead acetate solution. After varying periods of immersion, roots were cut off, rinsed in water and sections were examined in glycerine and ammonium sulphide. After 2½ hours in the lead solution, transverse sections showed a black precipitate of lead sulphide in the short unsuberised cells of the exodermis, the blackening being especially marked in the substance of and just inside the outer tangential wall. There was no indication of any penetration into the long suberised cells.

After two days in the lead solution, the precipitate in the short cells was still more marked and the lead had also penetrated through these passage cells into the cortical parenchyma as shown by a darkening of the cellulose walls as far as the endodermis. (Pl. III, figs. 1, 2, 3.)

These experiments, repeated with 1 per cent. Magdala red in place of the lead solution, gave similar results. After three days in the dye, sections were washed in 1 per cent. sodium bicarbonate solution and mounted in glycerine. The dye penetrated the short cells, but showed no signs of any entry into the suberised cells. This continued absorption by means of passage cells was evident to some extent over the whole length of root, but in the older parts substances accumulate in many of the short cells, which appear to prevent their functioning as passage cells. The lead or dye was seen to have entered these old cells, but usually did not penetrate through them into the cortex to any appreciable extent.

In the case of *Hyacinthus orientalis*, plants were grown in tap water, and when the roots were well developed some of them, whilst still attached to the bulb, were transferred to 1 per cent. lead acetate solution. After 20-30 hours in the lead solution, roots were cut off,

rinsed in water and treated with ammonium sulphide in glycerine. The roots presented a speckled appearance, which was seen especially well on examination with a lens (Pl. III, fig. 4). The black dots were most numerous in a zone about 2-3 cm. back from the root apex, but were present in smaller numbers and less evenly distributed as far back as the roots were in the solution—that is, to within about 1.5 cm. of the base of the roots with a total length of 14-16 cm. Microscopical examination showed the black dots to be short cells of the exodermis, in which lead sulphide was precipitated. In the region extending to about 1.3 cm. back from the apex, the passage cells were not conspicuous as none of the exodermal cells were suberised, but at a distance of 3 cm., the lead had entered the short cells and passed through them into the cortex, but had not penetrated the long cells, which at this level were suberised. At a distance of 7 cm. there was less penetration of the cortex beyond the passage cells and in still older parts the lead passed no farther than the passage cells themselves. Sections of this older region stained in Sudan III showed that failure of the lead to penetrate beyond the passage cells was due to an irregular fatty deposit over the inner tangential walls of these cells (Fig. 18).

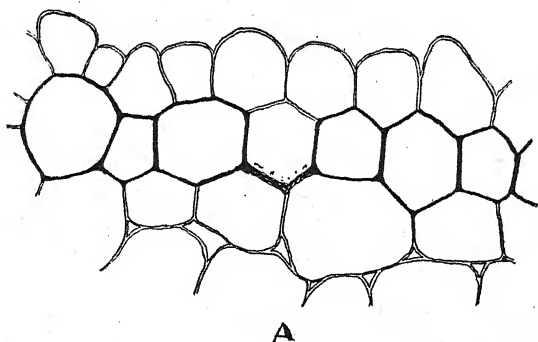
These experiments show that solutes of the nature of lead or dyes are unable to cross the suberin lamella of exodermal cells, but that where passage cells are present, substances may continue to enter the root above the level of development of the exodermis. These passage cells may, however, become obstructed in older parts of the root by the precipitation of substances in the cell, some of which may be of a fatty nature.

In *Vicia Faba* no suberised exodermis is developed, but as the root ages, the outer layers of cells turn brown and fail to give a blue colour with iodine and zinc chloride. In absorption experiments, these brown walls are found to be relatively impermeable.

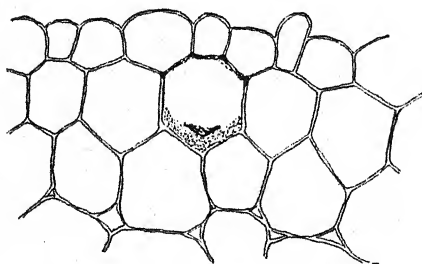
#### 7. SEASONAL VARIATION IN THE ROOT

In the literature there are numerous references to the fact that the length of the absorbing zone of a root varies with external conditions and is longest under conditions of moisture and temperature suitable for root growth. Most of these refer to the distance from the root apex at which the suberised cells of the exodermis and endodermis are developed, but in addition to these several definite cases of seasonal "elimination" of the typical absorbing zone have been described.

Harris (5) working on the activity of apple and filbert roots in Oregon and British Columbia describes the new rootlets in the spring as white and watery in appearance, whilst, at the approach of low winter temperatures, they "apparently lose water, shrivel in thickness and take on a hard brown skin." On the return of more favour-



A



B

Fig. 18. *Hyacinthus orientalis* ( $\times 300$ ). A. Root 16.7 cm. long. Section 12.4 cm. from the apex, stained with Sudan III, showing fatty deposit in the inner tangential wall of the passage cell. B. Lead sulphide in a passage cell. No penetration into the cortex beyond owing to deposits, which block the passage cell.

able conditions, the brown roots recommence growth "either by starting to extend their tip, send out side shoots or both." Under submerged conditions, he finds that only the larger roots survive, the smaller ones turning black and dying. Harris only describes the



external appearance of the roots, but it appears to be a case similar to that followed in the submerged roots of willow in this work. Trees of *Salix fragilis* ( $\times$  *pentandra*) growing on the border of ponds near Leeds, produced much branched masses of roots in the water of the type described as "queues de renard" by Daniel<sup>(2)</sup> and Bondonis<sup>(1)</sup>. During the summer from May onwards, the roots are tipped by a turgid, pink or white region, but in the autumn the brown colour of the older part of the root encroaches on the pink tip until the whole root appears brown by about the middle of December. This condition persists through the cold season until new growth recommences towards the end of March. This takes place in the larger roots by a rupture of the outer brown layer and continued apical growth, but this becomes less vigorous in the finer roots, whilst the finest of all do not appear to have sufficient vitality to break through the brown skin and simply blacken and die. Numerous new branch roots also appear in the spring (Pl. III, fig. 5).

In structure, the pink or white growing regions have a primary endodermis and the exodermal layer is unsuberised whilst the brown parts have a tertiary endodermis with or without passage cells and a suberised exodermis. In the spring and early summer growth is rapid, but as this becomes slower towards the end of the year, the typical absorbing zone is encroached upon by the progress of suberisation. Longitudinal sections of the roots in the winter condition show that not only are the suberised exodermis and secondary endodermis developed to within a short distance of the apical meristem, but that the elimination of the absorbing zone is completed by the impregnation of the walls of the root cap cells and those of the intervening piliferous layer with fatty and lignin-like substances. So that in the winter condition, the root apex is completely enclosed in a fatty and lignified covering, which extends from the root cap to the region where the typical suberised exodermis is developed. This crust or covering is cracked in one or several places at the commencement of growth (Fig. 19) and the new region of root usually swells to a greater diameter than the brown region of the root from which it emerges (Fig. 20). Bondonis<sup>(1)</sup> states that in submerged roots of willow, etc., the hypodermal layer is not suberised as in the case of typical exodermis formation, this being replaced by a suberisation of the superficial cells, but in the case of *Salix fragilis* in this work, the submerged roots always had a typical exodermis, though the walls of the superficial layer were usually suberised and lignified as well. The closure of the absorbing zone

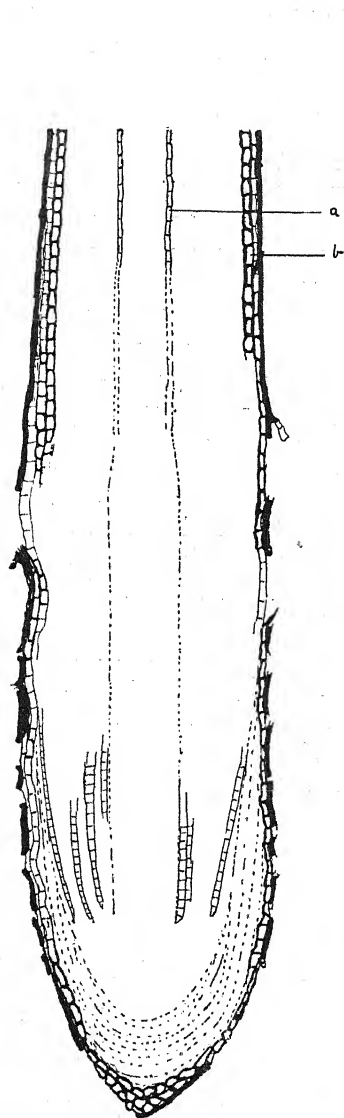


Fig. 19.

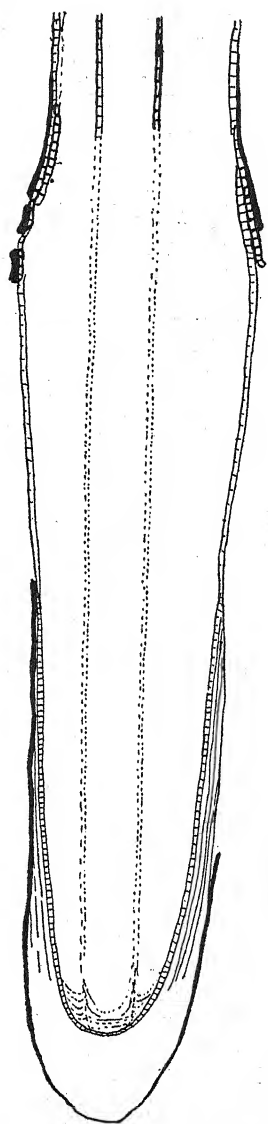


Fig. 20.

Fig. 19. L.S. *Salix fragilis* ( $\times$  *pentandra*) ( $\times$  46). Water root on March 25th, 1926, showing cracking of the crust, which envelops the apex during winter. Stained in Sudan III. *a*, suberised endodermis; *b*, suberised exodermis.

Fig. 20. L.S. *Salix fragilis* ( $\times$  *pentandra*) ( $\times$  46). Water root on March 25th, 1926, stained in Sudan III, showing the swollen appearance of the new region of the root as it breaks through the winter envelope.

during conditions unsuitable for growth is probably of fairly common occurrence. Plaut(11) mentions that it takes place in many forest trees and it is also described in alder (Wolpert(24)) and vine (Kroemer(6)). Plaut describes the process in Gymnosperms where he finds examples in all groups other than the Abietineae and Gnetales. In Gymnosperms the suberised cells of the superficial layer may be connected with the secondary endodermis by similar impregnation of the walls of an intervening layer of cortical cells.

In the Monocotyledons, Müller(9) describes the closure of the absorbing zone by a somewhat similar process in *Convallaria majalis*. In the late summer and autumn the roots cease to grow and changes take place in the outer part of the root apex which "apparently completely prevent the passage of dissolved substances and eventually that of water also to some extent." The outer layer of cells of the root cap and a short zone of epiblem cells become lignified and suberised and connect up with the exodermis, so enclosing the root tip in a fatty covering. According to Nicolai and van Wisselingh, the exodermis of *Convallaria majalis* is of the type with short, unsuberised cells, so that the closure may not be as complete in this case as in *Salix*, but at any rate Müller finds that roots enclosed in this way do not grow again and are replaced on resumption of growth by new roots from the rhizome or branch roots. Examples of *Funkia* roots taken from the soil in winter or from dry soil in June showed a similar enclosure of the apex in a fatty covering to that described by Müller for *Convallaria*, whilst roots from moist air over water, which had been growing rapidly, had a typical absorbing zone between the root cap and the development of the exodermis. In these cases of suberisation and lignification of the walls of the superficial and root cap cells the impregnation is more generally distributed in the wall and not confined to a definite lamella as in the normal suberisation of exodermal and endodermal cells, and also the fat is more easily removed from the walls. The difference probably lies in the fact that the development of the typical suberin lamella takes place in relatively young walls, whilst this process may take place when the walls are more mature. Kroemer distinguishes this later process as "Metacutisierung" (6).

A similar enclosure of the root apex by a fat impregnated layer was seen on one or two occasions in *Hyacinthus*. A number of bulbs were not planted at the proper time and were stored in the laboratory at a somewhat high temperature and when ultimately planted later

in the season many of them failed to make any root growth. Sections through the root initials showed these to be completely enclosed in a fatty covering, through which the root was apparently unable to break. As lateral roots are not known in *Hyacinthus*, this resulted in no root development.

#### 8. CONCLUSIONS

In a previous paper on the absorbing region of the root, the entry of water and salts is considered as taking place chiefly along the cellulose walls of the cortex as far as the endodermis and then across the endodermal protoplasts into the stele and it is obvious that on this or any view of root absorption, any changes in the walls exterior to the stele, which reduce their permeability, must of necessity reduce absorption. It was put forward by Meyer about 1880 that the cuticularised and corky parts of the membrane in the hypodermis, endodermis and superficial cells of the root would obstruct to some extent the passage of water soluble salts and soluble reserve substances across these cells and this conception is the basal idea in the work of Kroemer and Mylius on endodermis, exodermis, polydorm and cork and is generally accepted in the literature. De Lavison (7) tried to test this experimentally and found that salts and dyes were stopped by the suberised exodermis of *Hyacinthus* and in younger regions substances such as iron salts and certain dyes, which did not penetrate protoplasm, were also stopped by the suberised radial walls of the endodermal cells. Experiments in the present work again give no indication of the passage of salts or dyes across suberised membranes so that the distribution of suberisation may be used as an indication of the delimitation or curtailment of the absorbing region of the root. In the discussion of absorption it was stated that under favourable conditions water would be drawn across the endodermal protoplasts by osmosis, the difference in osmotic concentration between the stelar and external solutions being maintained by the liberation of solutes in the differentiation of new vascular elements during the process of growth. During the early part of the growing season there may be fluctuation of pressure in the vascular system during the 24 hours owing to the fact that during the day the loss of water by transpiration may exceed the gain due to entry at the root, whilst at night the reverse is the case. Either of these conditions, however, would be in accordance with continued entry of water across the endodermis, and the maintenance of a typical absorbing region in the root. Later in the

season, when more leaves are expanded, there is the probability that the excess loss of water during the day would fail to be compensated by entry at the root during the night and under these conditions, though water would still continue to be drawn across the endodermis, the tendency would be for the growth rate to fall off, whilst lignification and suberisation would continue and probably be accelerated since these processes appear to be due in part to oxidation and drying. McDougall (8), studying growth of the roots of forest trees in Illinois, found that in 1914 root growth stopped in August due to drought, recommencing with the September rains, whilst in 1915 there was no summer drought and no cessation in root growth and he regards water supply as the chief governing factor. In the observations of Harris (5) on apple and filbert and the present observations on water roots of *Salix*, growth appears to be stopped by the advent of low temperatures. But, whether root growth completely stops or not, under less favourable conditions the suberised layers of endodermis and exodermis encroach upon the typical absorbing region. The extent to which absorption is curtailed by this process will vary in the first place with the type of endodermis and exodermis characteristic of the particular plant, being almost complete in types with a complete secondary or tertiary endodermis, e.g. *Rumex acetosella*, or completely suberised exodermis, e.g. *Salix fragilis*, and only partial in types with permanent passage cells in endodermis and exodermis, e.g. *Funkia* and *Hyacinthus*. In the case of plants with passage cells in the endodermis, water may be drawn in either direction across the protoplasts of the passage cells according to the relative suction pressures of the cells on either side so long as the protoplasts retain their semipermeability. If, however, the protoplasts become more permeable with increasing age, there will be a possibility of actual flow across the endodermis of water with dissolved organic and inorganic solutes and probably increased permeability of this kind may account for phellogen formation external to a primary endodermis or a secondary or tertiary one with passage cells, e.g. *Philodendrom erubescens* (aerial root) and *Monstera deliciosa* (16).

In the case of the exodermis with passage cells, suberisation of the radial walls prevents inward diffusion such as normally takes place in the absorbing zone, but in the young stages water may be drawn across the passage cells by the suction pressure of the cortical cells. With increasing permeability of the protoplasts of the passage cells, there is again a possibility of flow, but actually in these cells

the breaking down of the protoplasts is accompanied by deposits in the cells which appear to render them relatively impermeable.

The seasonal enclosure of the root apex in a complete fatty covering is a further extension of the process by which the suberisation of the endodermis and exodermis encroaches on the absorbing zone. This metacuticularisation of superficial walls, in association with a completely suberised exodermis of the *Salix* type, gives the most complete example of elimination of the absorbing zone of a root.

#### 9. SUMMARY

1. A few plants belonging to different types of root system are examined in order to determine in what way and to what extent the absorbing zone is delimited in older parts.
2. The endodermis develops close behind the meristem and appears to be associated with vascular differentiation. The accumulation of evidence establishes the fatty impregnation of the Casparian strip. Comparison of the behaviour of the tertiary endodermis in Fern and Angiosperm suggests that the suberin of the secondary lamella is laid down in a basal wall substance in the Angiosperm. Investigation by microchemical reactions is also suggestive on this point.
3. The distribution of different types of endodermis in the plants under consideration is outlined. The death of the cortex and the absence of cork external to a completely suberised endodermis is quoted as evidence for impermeability of suberised membranes.
4. The development of the exodermis in *Funkia* is followed and the mature exodermis is shown to consist of long suberised and short unsuberised cells. The microchemical reactions of the suberised exodermal cells agree with those of tertiary endodermal cells, except that no resistant layer comparable with the Casparian strip is present. Other types of hypodermis are compared with the *Funkia* type.
5. The results are given of macrochemical analysis of the exodermis of *Hyacinthus*. The ratio of  $\frac{\text{Normal and Oxy-Acids}}{\text{Chloroform Soluble Fat}}$  is intermediate between that given by potato cork and the tertiary endodermis of *Potamogeton*.
6. Experiments with lead salts and dyes show that the suberised cells of the exodermis are not permeable to these substances, but the unsuberised cells act as passage cells. They may become blocked in older regions.

7. The distance behind the meristem at which suberisation of membranes appears, varies with the season. Examples from diverse groups of plants show that the typical absorbing zone may be eliminated by the development of the suberised exodermis and endodermis to within a short distance of the root apex and the completion of the closure by fatty impregnation of the walls of superficial cells.

8. In conclusion, absorption under different moisture conditions is discussed in relation to the different types of endodermis and exodermis and in relation to seasonal variation in the extent of the absorbing zone.

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## EXPLANATION OF PLATE III

- Fig. 1. T.S. *Funkia ovata* root after 2 days in 1 % lead acetate solution. The lead, precipitated as lead sulphide, shows penetration into the passage cells of the exodermis, but not into the suberised cells. The lead has passed through the passage cells into the walls of the cortical cells beyond. ( $\times 280$ .)
- Fig. 2. T.S. *Funkia ovata* root (same section as Fig. 1) showing penetration of the lead along the cortical walls as far as the endodermis. ( $\times 112$ .)
- Fig. 3. T.S. *Funkia ovata* root (same section as Fig. 1) showing the inward movement of the lead, stopped at the endodermis. ( $\times 262.5$ .)
- Fig. 4. *Hyacinthus orientalis*. Strips of exodermis about 8.0 mm. from the apex of a root, after 2 days in 0.5 % lead acetate solution. ( $\times 21$ .)
- Fig. 5. Water roots of *Salix fragilis* ( $\times$  *pentandra*) in June, showing the white regions of new growth and the very slight growth of finer roots.





Fig. 1.

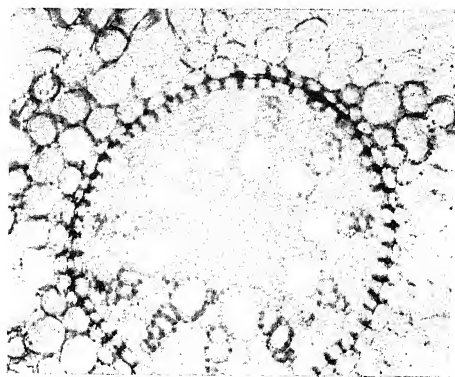


Fig. 2.



Fig. 3.

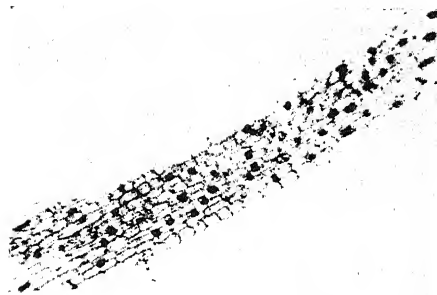


Fig. 4.

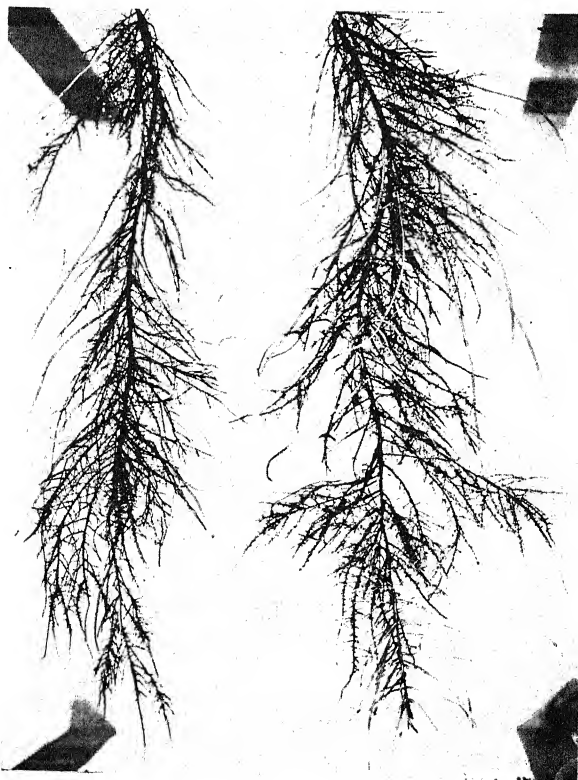
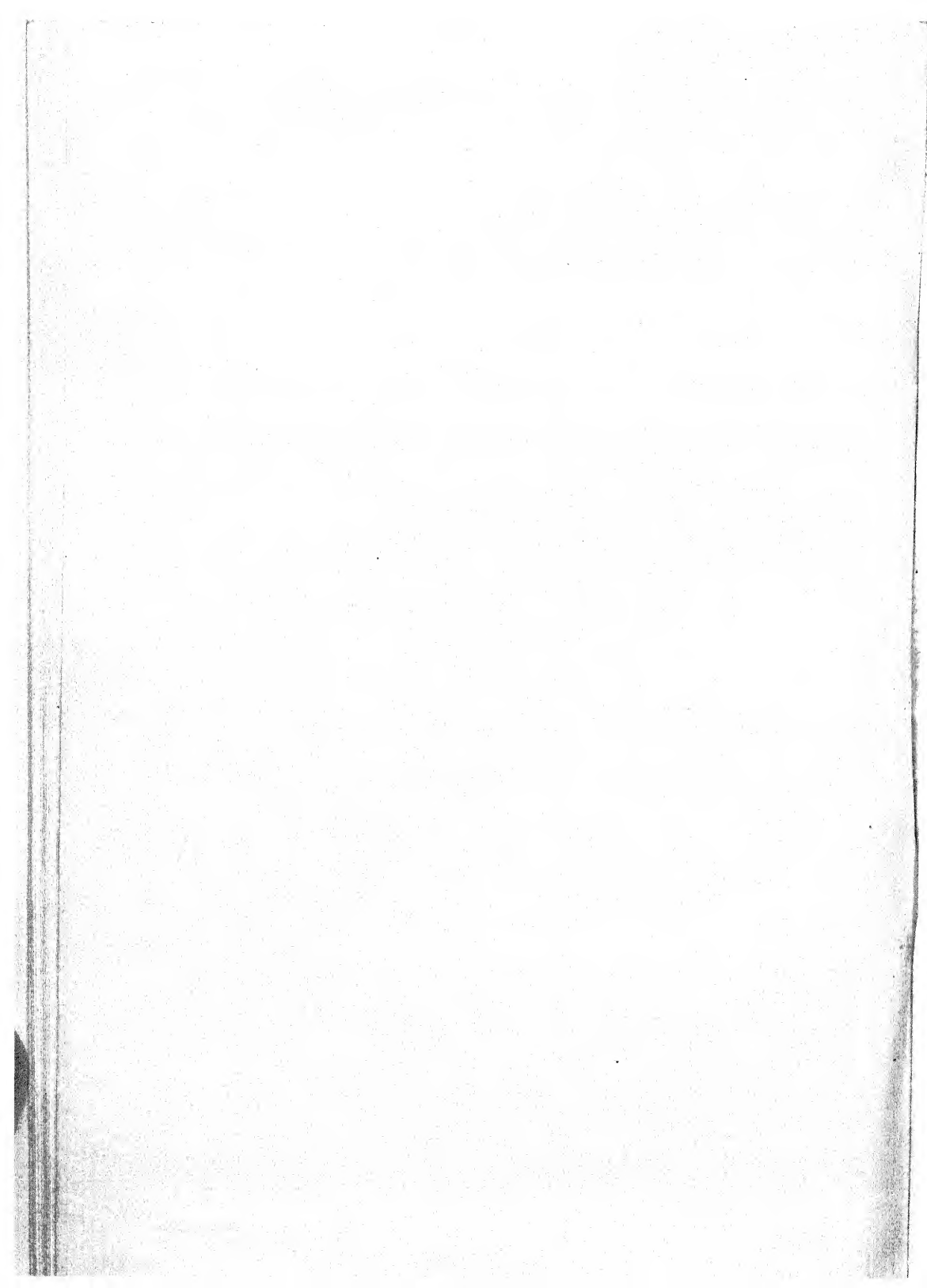


Fig. 5.



## ILLUSTRATIONS OF CARPEL POLYMORPHISM. II

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(With 62 figures in the text.)

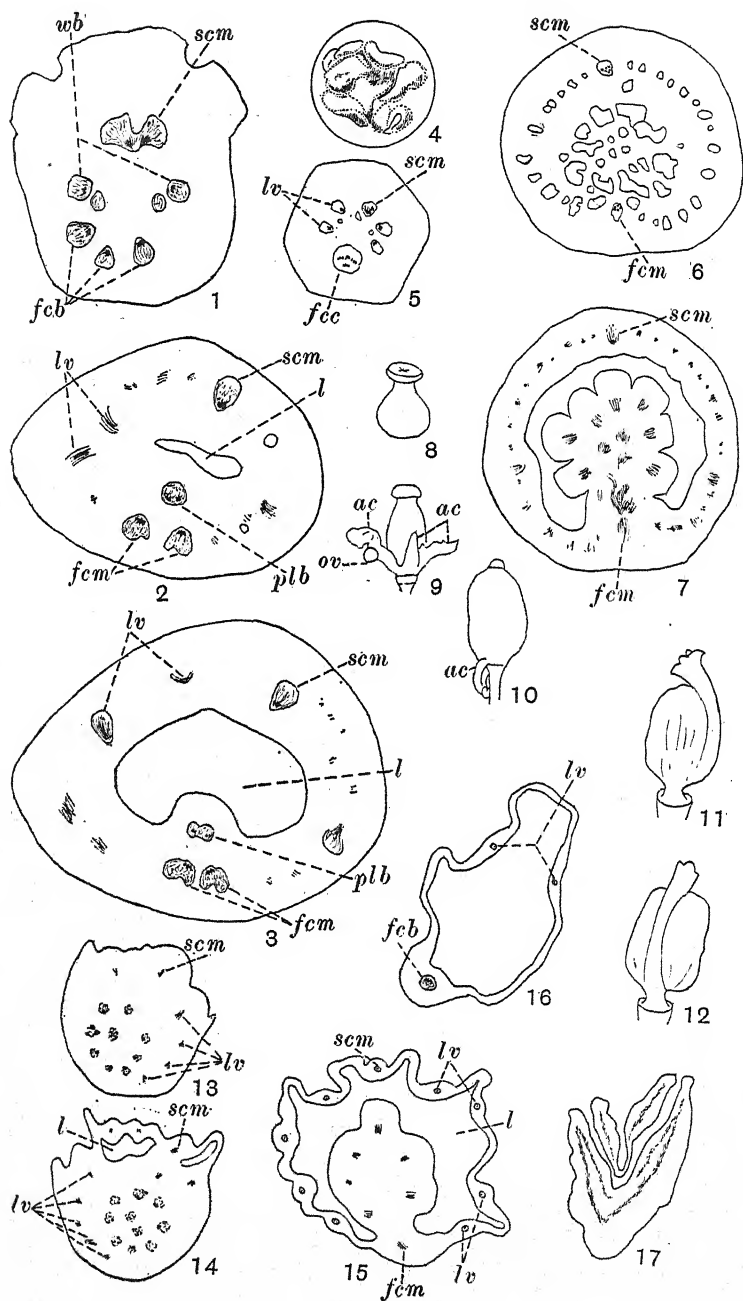
### BERBERIDACEAE

THE family of the Berberidaceae, according to the former view that the carpel is monomorphic, is characterised by having an ovary composed of a single carpel. As was, however, shown in an early statement of the polymorphic theory<sup>1</sup> one genus belonging to this family—*Epimedium*—affords the clearest example of the polymorphic condition, the ovary being obviously not monomerous, but dimerous. It was pointed out also that *Jeffersonia* was evidently constructed upon a similar plan. Examination of other genera (*Podophyllum*, *Berberis* and *Mahonia*, *Leontice*, *Nandina*) has now made it abundantly clear that this dimerous condition is found throughout the whole family. There is, consequently, no longer need to formulate some plausible explanation of the solitary terminal carpellary leaf for here it clearly does not exist. It is hoped in a later account to show that in other families in which this anomaly has been believed to occur the 1-carpel interpretation is similarly at variance with the evidence afforded by various structural features.

*Epimedium*. The structure of the gynoeceium in this genus has already been fully described<sup>2</sup>. The main features are recalled here merely for comparison with the other genera dealt with below. The ovary is composed of two carpels, one of the valve type, small, sterile, and destitute of style and stigma, the other, large, semi-solid and fertile, with style and capitate stigma. The membranous ovary wall shows an obliquely vertical furrow on each side indicating the lines of junction of the two members. The wall here is thinner than elsewhere and destitute of vascular tissue, the venation system of both carpels falling short of their boundaries. The dry fruit dehisces along the two sutures when ripe; the sterile valve is then shed exposing the seeds still attached to the inner face of the persistent, larger carpel.

<sup>1</sup> Carpel Polymorphism, I. *Annals of Botany*, 39, pp. 131-3, Figs. 4-6. 1925.

<sup>2</sup> *Loc. cit.*



*Podophyllum peltatum* L. (Figs. 1-4). The much larger gynoecium of *Podophyllum* has the vascular system particularly well developed. Transverse sections through the ovary base show on the one side the midrib and wing bundles of the expanded, sterile, valve member, and on the opposite side the bundle system of the consolidated, fertile carpel (Fig. 1). This latter system shortly becomes concentrated into the twin strands of the midrib and the double bundle of the placenta (Figs. 2, 3). At this and higher levels the numerous lateral veins of the valve are seen cut obliquely. As is commonly the case with the consolidated carpel type the ovules, which extend almost to the top of the placental cushion, are borne in several rows. The only other feature calling for comment is the stigma which appears as a series of convolutions crowning the ovary, and so intercoiled as to obscure any definite relation to the two carpel members (Fig. 4).

Figs. 1-17. Berberidaceae. 1-4. *Podophyllum peltatum* L. 1. Transverse section of the ovary below the level of the loculus. In the upper half are seen the midrib, lateral (wing) bundles and two smaller strands belonging to the sterile valve carpel, on the lower side the bundles destined for the fertile carpel. 2. The same at the level of development of the loculus; the fertile carpel shows twin bundles constituting the midrib and a double bundle in the placenta. 3. The same at a higher level with the placental cushion now well developed. 4. The ovary apex viewed from above showing the stigma. 5-8. *Berberis vulgaris* L. 5. Transverse section of the ovary taken through the extreme base. Towards the upper side, the midrib and secondary veins of the sterile valve; opposite, the large cord (midrib) of the fertile carpel. 6. The same just below the level of origin of the loculus. Above, the midrib, and at the periphery, the secondary veins of the valve; below, the midrib of the fertile carpel; in the centre, the numerous strands given off from the midrib which traverse the placenta and serve the ovules. 7. The same at the ovule-bearing level. 8. The gynoecium showing the circular form of the stigma. 9, 10. *Berberis* (*Mahonia*) *Aquifolium* Pursh. The gynoecium and accessory carpel structures in the flowering (9) and fruiting (10) stage. 11-17. *Leontice Chryso-gonum* L. (*Bongardia Rauwolfii* C. A. Mey.). 11, 12. Two views of the gynoecium. 11. Side view of the two carpels. 12. Face view of the solid, fertile carpel. 13-17. Transverse sections of the gynoecium at successively higher levels. 13. The ovary below the level of the loculus. The midrib and lateral veins of the sterile, valve carpel are seen cut longitudinally as they run out to the periphery; the strands destined for the fertile carpel are not yet fully differentiated. 14. The same, as the two carpels begin to separate along their inner faces to form the loculus. 15. The same, in the ovule-bearing region. 16. The same, above the ovule-bearing region. 17. The stigma after the stylar canal has opened on to the surface; the larger lip is formed of the median portion of the solid carpel, the smaller, of the lateral portions of the same connected by a narrow bridge of tissue derived from the valve carpel. The regions corresponding to the solid carpel are abundantly supplied with vascular tissue.  
ac, accessory carpels; fcb, fertile carpel bundles; fcc, fertile carpel cord; fcm, fertile carpel midrib; l, loculus; lv, lateral veins of the valve carpel; ov, ovule; plb, placental bundle; scm, sterile carpel midrib.

*Berberis vulgaris* L. (*Euberberis*), *Berberis Aquifolium* Pursh. (*Mahonia*) (Figs. 5-10). The ovary in *Berberis* is constructed upon the same plan as that of *Podophyllum*, but here the ovules, similarly borne in several rows, are restricted to the basal region of the placenta. At the ovary base the two carpel midribs are easily distinguished (Fig. 5), but the repeated branching of the fertile vascular cord (Fig. 6) to form the several strands which later become condensed in the placental cushion to correspond with the number of ovules (Fig. 7) renders the construction at this intermediate level less clear (Fig. 6) than in the preceding genus; but as the loculus level is reached the bicarpellary ground plan is again clearly apparent (Fig. 7). The whole of this placental vascular tissue is used up in supplying the ovules but the midrib is continued to the top, as well as the venation system of the valve carpel. As the stigma region is approached the two systems give rise to a ring of small equal bundles, a reflection of this symmetrical distribution being seen in the circular form of the stigma, the soldering of the two carpels being here complete to the top (Fig. 8).

Occasionally individual branches on a *Mahonia* bush are found bearing flowers with an extra whorl of floral structures between the androecium and the gynoecium proper. I am indebted to Miss D. Milner Brown for some specimens showing this peculiarity in the flowering (Fig. 9) and fruiting (Fig. 10) stages. These supernumerary structures, which vary in number, are obviously carpellary in nature. They may be separate or partially united; in the latter case they form a lobed "collar" round the normal ovary. The separate structures, in their most reduced condition, are thread-like with a capitate stigma and correspond each to a single carpel. More often they are plate-like and stigmatic along the extent of the upper edge. From the present point of view these latter structural forms are of considerable interest. They are unable, doubtless owing to spatial relations, to form so many individual, closed ovaries, but they are, nevertheless, evidently bicarpellary for they show two separate vascular cords. In one of these exceptional flowers one of these accessory organs was found to be as well developed as the normal ovary to which it was adherent, producing in this way the appearance of "twinning." Although distorted in form the accessory ovary contained ovule-like structures.

*Leontice Leontopetalum* L., *L. Chrysogonum* L. (*Bongardia Raewolfii* C. A. Mey.) (Figs. 11-17). *Leontice* resembles *Epimedium* in that the bicarpellary construction is indicated in the outward appear-

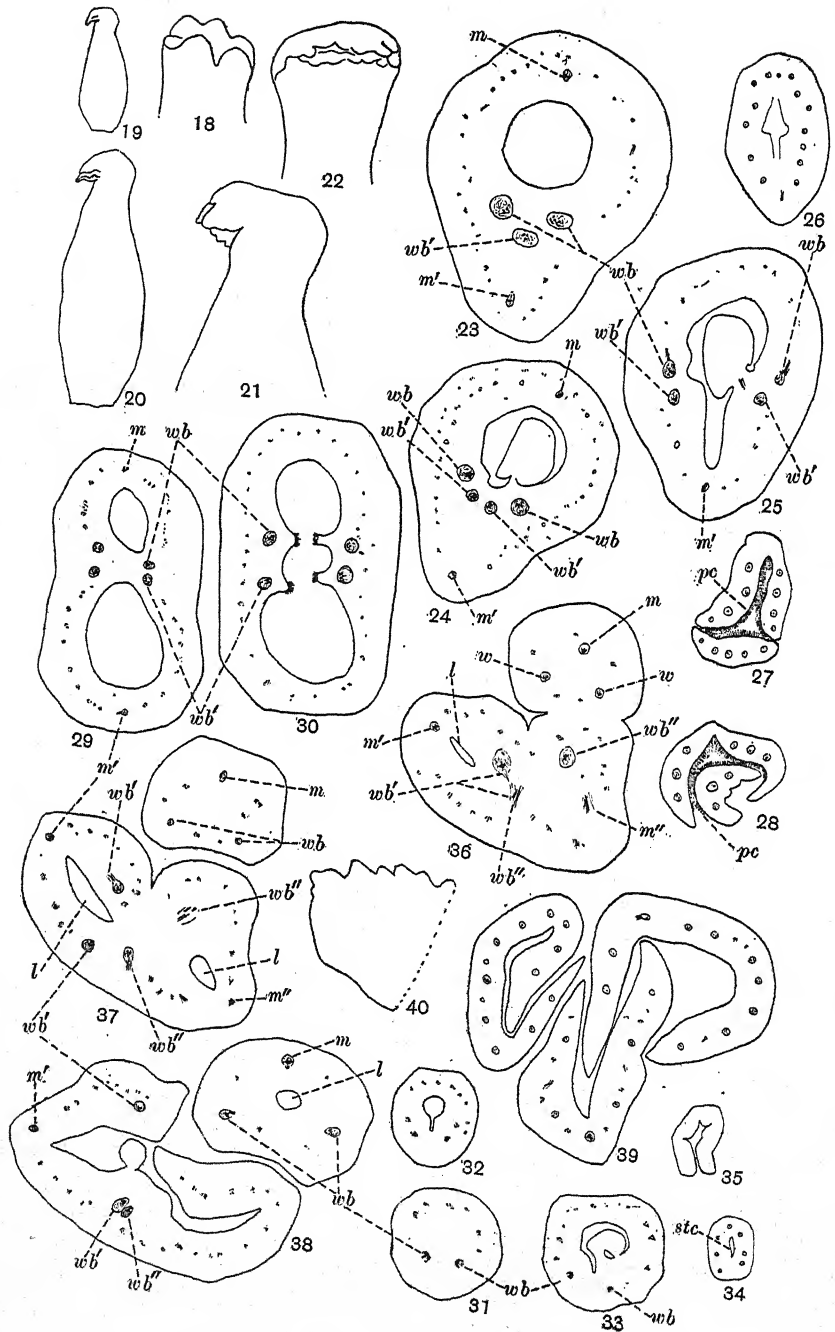
ance of the gynoeceium, but the size relation of the carpels is reversed in the two cases. For whereas in *Epimedium* the fertile member is semi-solid and has considerable lateral extension, the valve, in consequence, being small, in *Leontice* the fertile carpel is contracted to the completely solid form and is seen on one side of the ovary as a stout rib or column of tissue which is continued upwards as the style (Figs. 11, 12). The rest of the wall, which is thin and membranous, and served by a series of fine secondary veins, is formed by the large valve carpel (Fig. 15). In the style column, however, the valve carpel component becomes greatly reduced until at the stigma level it is represented by a short non-vascular bridge connecting the incurved edges of the solid member.

In both species the dry fruit wall sooner or later ruptures, becoming irregularly torn, but in neither case is there true dehiscence. This is no doubt attributable to the extreme paucity of such mechanical elements<sup>1</sup> in the vascular bundles as would be capable of setting up the degree of tension which one assumes to be required to bring about dehiscence at some particular locus.

In a cross-section taken through the ovary base of *L. Chrysogonum* strands are seen running out from the residual vascular tissue to the periphery round the greater part of the circumference. These become the midrib and secondary veins of the sterile valve member (Fig. 13). At a slightly higher level the loculus makes its appearance as a periclinal split (Fig. 14) between an arc of thin wall formed of the valve carpel and a bulky mass of tissue which separates more and more from the valve wall as it becomes differentiated into the solid carpel with midrib and placental bundles (Fig. 15). The ovules, which are confined to the basal portion of the placenta, arise on several radii. The whole of the vascular tissue in the placenta is utilised in their formation so that above the ovule level the midrib alone persists (Fig. 16). In the valve carpel, on the other hand, the midrib is weakly developed and soon comes to an end, a pair of lateral veins alone persisting towards the apex.

As the stigma level is reached the stylar canal opens out on to the surface right and left of the fertile cord and at some distance from it. Of the two lips thus formed the smaller, in its middle region, is destitute of vascular tissue. This region represents the last remnant

<sup>1</sup> In the flowering stage these consist of no more than some two or three very small vessels in each vein in an ovary wall which, in *L. Chrysogonum*, mostly shows but one single thin cell layer between the inner and outer epidermis, and in *L. Leontopetalum*, generally not more than three or four similar layers.





of the valve carpel. The margins of this lip and the whole of the larger one, which together represent the solid carpel, are well supplied with vascular elements (Fig. 17).

*Nandina domestica* Thunb. (Figs. 18-40). The occurrence of certain variations from the normal in the gynoeceium of *Nandina* renders this genus one of the most interesting in the family from the present viewpoint. The ovary at first is radially symmetrical with erect stigma lobes (Fig. 18) but as development proceeds it becomes markedly bilateral, the apical portion being bent to one side at a right angle so that it roughly resembles the head and beak of a bird (Figs. 19, 20). This zygomorphism is due to unequal growth in length of the two carpels, the longer forming the upper, the shorter, the lower lip of the "beak." Here we have developed but slightly that inequality in size between the two component carpels which is exhibited in such a much more pronounced form in *Jeffersonia*, *Leontice* and *Epimedium*. Both stigmatic lips show a number of blunt marginal lobes, reflecting probably the pattern of the lateral vein system (Figs. 21, 22). The most striking feature of the pistil, however, is that both carpels are of the valve type though generally one alone is fertile, this member being then more fully expanded as well as longer than the other (Figs. 23-28). Exceptionally, however, both may expand equally and both bear ovules (Figs. 29, 30). Ovaries of this type were observed by Citerne<sup>1</sup> and correctly interpreted, but he noted them merely as abnormalities and missed their significance in relation to the ordinary type. In these exceptional cases the ovary may show a furrow down each side along the line

<sup>1</sup> *Berberidées et Erythrospermées*, p. 28. Paris, 1892.

Figs. 18-40. Berberidaceae (continued). *Nandina domestica* Thunb. 18. Gynoeceium apex from a young bud, showing erect stigma lobes (highly magnified). 19. Whole gynoeceium at an older stage with the apex bent over to one side. 20. The same more highly magnified. 21, 22. Apex of the same still further magnified. 21. The "beak" in side view. 22. The same in face view. 23-39. All from transverse sections taken at successively higher levels. 23-28. From a well-developed ovary in which the two carpels are slightly unequal. 29-30. From a well-developed ovary of two equal carpels. 31-35. From a poorly-developed ovary. The infertile carpel is much reduced and destitute of vascular tissue; the second loculus is represented by a narrow channel leading out of the main chamber. 36-37. From an ovary composed of three equal valve carpels which ultimately become disunited. 40. Portion of the apex of one of the three carpels cut off and laid flat showing the lobing of the stigmatic margin.

*l*, loculus; *m*, *m'*, *m''*, midrib bundle; *pc*, papillose cells of the stigma; *sc*, stylar canal; *wb*, *wb'*, *wb''*, wing bundles.

of the conjoined edges thus producing an effect as of "twinning" of the gynoeceium. Both carpels expand to enclose a chamber and the ovary may then for a short distance at the base be bilocular (Fig. 29). But the loculi enlarge at once, the intervening tissue disappears, and the two become continuous. Where the partition previously appeared there are now to be seen four projecting points, two in close juxtaposition on each side of the now single extended loculus, each of these pairs developing hairs and representing the conjoined margins, on the one side and the other, of the two carpels. The carpels show a midrib, two prominent wing bundles and a number of smaller veins running more superficially (Fig. 30). In all cases the secondary veins gather together towards the apex into a smaller number of larger bundles (Figs. 26-28), and their distribution possibly determines the lobing of the stigmatic margins referred to above (Figs. 21, 22). In poorly developed ovaries the infertile carpel may be reduced to such an extent as to show no vascular elements at all, only undifferentiated tissue occupying the area between the wing bundles of the fertile member<sup>1</sup> (Figs. 31-33). In these cases the ovary is unilocular throughout, the main chamber representing the loculus of the fertile carpel. From this there opens out on a radius between the wing bundles a narrow bay or channel which is all that the reduced carpel is able to achieve in the way of a loculus (Fig. 32). I was for long puzzled by this indentation in this type of ovary until I succeeded in obtaining more favourable material in which the bicarpellary ground plan was unmistakable.

Occasionally tricarpellary ovaries occur, each of the three carpels being of the valve type and enclosing a loculus (Figs. 36-39). The fitting together of three members seems, however, to present some difficulty. One of the three may sooner or later become disjoined from the other two and suffer some distortion. Or all three may become free and assume the form of folded leaves, the disunited portions dovetailing one with another (Fig. 39).

The evidence furnished by the series of types described above affords the clearest proof that the former monocarpellary interpretation of the gynoeceium is untenable even apart from the fundamental difficulty involved in the conception of a terminal foliar organ.

A point deserving of notice is that when two placentae belonging to different carpels bear each an ovule, as undoubtedly is occasionally

<sup>1</sup> A small group of a few xylem elements seen in one case close to one of the wing bundles, which came to an end below the level of the loculus floor, possibly represented a last remnant of the residual vascular tissue from which the second carpel cord is differentiated in better developed ovaries.

the case, we have a genuine instance of *parietal placentation in a syncarpous gynoeceium*, a condition which in the light of our present knowledge must be regarded, contrary to received opinion, as of rare occurrence. This feature of his exceptional cases was appreciated by Citerne but he failed to perceive that these very cases provide us with a most valuable clue to the plan of construction of the ordinary type of gynoeceium. For from these instances of equal carpel development we can trace a series showing increasing disparity in size between the two carpel members and consequent asymmetry of external contour as we pass from the various *Nandina* forms to *Epimedium*, *Leontice* and *Jeffersonia*. In marked contrast to this series is the *Berberis-Podophyllum* group, for in these genera the two carpels although dissimilar in type are of equal height and so evenly united that the mature gynoeceium is radially symmetrical.

On the basis of the carpel characters the genera dealt with above can be arranged in series thus:

*Nandina*. Rare variation. Carpels 3, all of the valve type and equal.

*Nandina*. Occasional variation. Carpels 2, both of the valve type, and both equal and fertile.

*Nandina* type. Carpels 2, both of the valve type, slightly unequal, one only being fertile.

*Nandina*, depauperated. Carpels 2, both of the valve type, the infertile one much reduced and destitute of vascular tissue.

*Berberis* (including *Mahonia*) and *Podophyllum*. Carpels 2, unequal, one of the valve type and sterile, one solid and fertile. Ovary radially symmetrical.

*Leontice* (including *Bongardia*). Carpels 2, unequal, one of the valve type and sterile, one solid and fertile. Ovary bilaterally symmetrical.

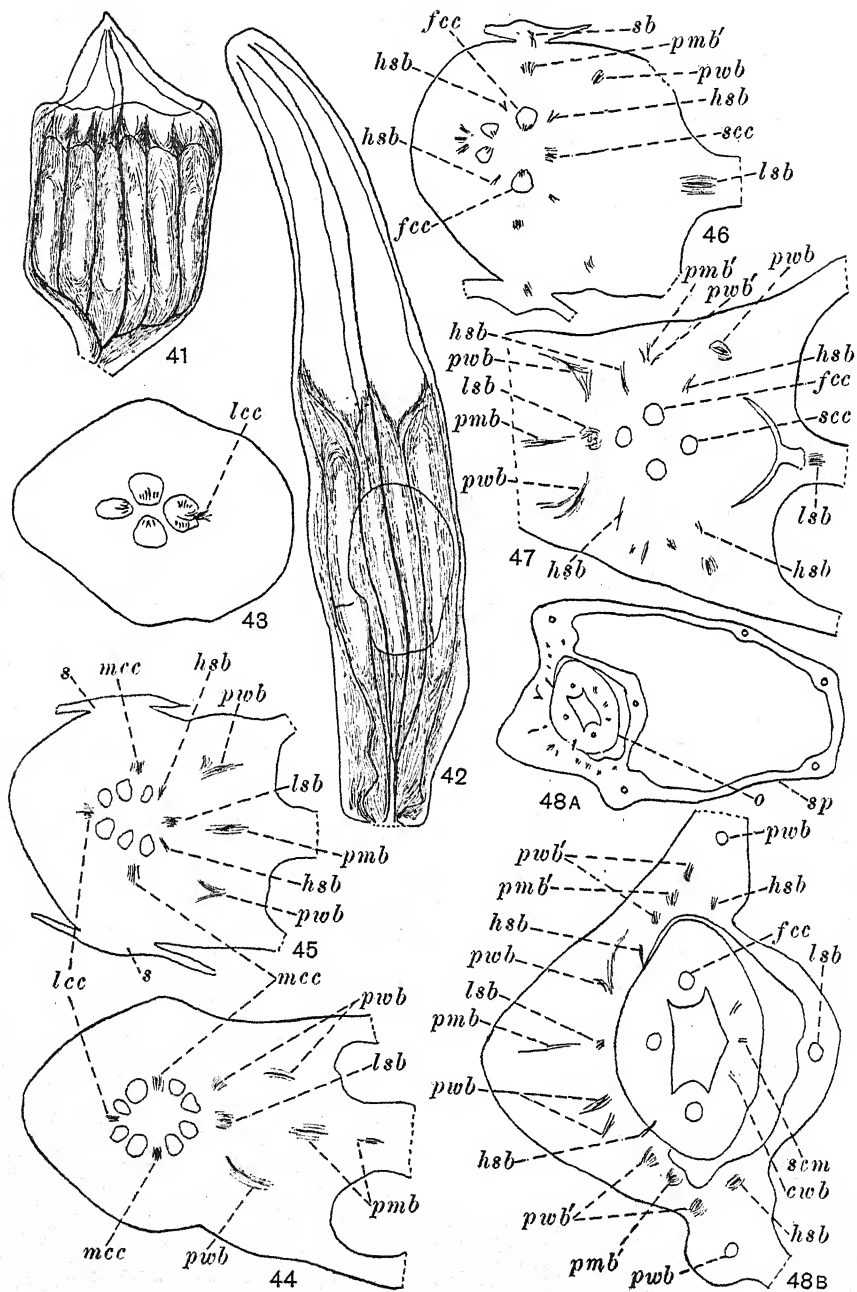
*Epimedium* and (probably) *Jeffersonia*. Carpels 2, unequal, one of the valve type and sterile, one semi-solid and fertile. Ovary bilaterally symmetrical.

#### FUMARIACEAE

*Ceratocarpus* (*Corydalis*) *palaestina* Boiss. (Figs. 41, 42). The two or three species included in the section *Ceratocarpus* are of extreme interest on account of their heteromorphic fruits, urn-shaped in the first few flowers (Fig. 41), silique-like in all the rest (Fig. 42). Examination of material of *C. palaestina* recently sent to me by Mr J. E. Dinsmore from Jerusalem has shown that the lower fruits owe their shape to two short and broad valves, and two solid carpels, *not*, as I had earlier suggested<sup>1</sup>, to four *similar* carpels.

In the many-ovuled species of *Corydalis* and in *Dicentra* the midribs of the two narrow, sterile, valve carpels give off a succession of lateral veins. The amount of xylem in the bundles is small and

<sup>1</sup> From such evidence as is afforded by descriptions and figures. See *Annals of Botany*, 37, p. 474 and Figs. 52, 53 (p. 477). The silique has narrow, long valves.



there is almost a complete absence of mechanical tissue. In both the fruit types of *C. palaestina*, which are 1-ovuled, the chief lateral veins (usually six in the urceolate form) all take their rise at the ovary base and almost at the same level. Both types differ from the fruits of other species not classed under *Ceratocarpus* in being what may be described as "two-storeyed," herein showing some resemblance to certain genera of the Cruciferae. Both also develop a large amount of mechanical tissue in the form of longitudinal bands which come to an end just above the junction of the two storeys, anastomosing at this level so as to form a horizontal girdle. These bands are associated with the vascular bundles. In the lower section of the urn-shaped fruits the chief lateral veins leave the main cord of the valve as a single trunk which runs out on either side horizontally,

Figs. 41-48. Fumariaceae. 41, 42. *Ceratocarpus* (*Corydalis*) *palaestina* Boiss. 41. A ripe urn-shaped fruit with the upper triangular portion entire but with one of the two lower valves removed, the remaining valve seen from the outside. 42. A whole immature fruit of the later-formed siliquoid type. Near the edge to the right and left the main cords of the two median solid carpels, the one on the left shows the branch given off to the solitary ovule. Between, the midribs and the strongest of the lateral veins of the two sterile valve carpels. 43-48. *Corydalis lutea* L. All from transverse sections taken at successively higher levels. 43. Flower stalk. The vascular mass on the right in process of giving off the composite cord for the spurred petal and superposed stamen. 44-48. Flower base. Part of the spurred (right) petal has been cut away, the cut edges being indicated by the interrupted portions of the contour line. 44. On the right the midrib and lateral veins of the spurred petal; nearer the centre and on the same radius the bundle of the lateral (whole) stamen. The compound cord for the left (unspurred) petal and whole stamen, and the two compound cords consisting of the conjoined bundles of a sepal and an inner petal are leaving the central ring on the left, and in the median plane back and front, respectively, and are seen cut longitudinally. 45. At the level of exertion of the two small median sepals. The two small strands destined for the two half stamens on the right are about to leave the central ring. 46. The conjoined bundles of the sepals and median petals have separated. The small bundles for the two half stamens on the left and the larger cords for the two median solid carpels and the right valve carpel can now be identified. The composite cord on the left has already given off the wing bundles of the petal but the midrib is not yet disjoined from the stamen bundle. 47. Differentiation of the petal, stamen and carpel bundles on the left follows the same course as that already completed on the right where the ovary surface is now exposed. 48 A. The ovary, now almost free, is enclosed on the right by the coherent bases of the stamen triplet, which in turn is encompassed by the spurred petal. On the left the tissues of the petal (unspurred), stamen triplet and ovary are still continuous. 48 B. Portion of the same more highly magnified.

*cwb*, sterile carpel wing bundle; *fcc*, fertile carpel cord; *hcb*, half stamen bundle; *lcc*, lateral conjoined cord of petal and stamen; *lsb*, lateral stamen bundle; *mcc*, median conjoined cord of sepal and petal; *pmb*, *pmb'*, petal midrib bundle; *pwb*, *pwb'*, petal wing bundle; *s*, sepal; *scc*, sterile carpel cord; *scm*, sterile carpel midrib; *sp*, spurred petal.

from which the individual veins then separate in succession at the same time, turning again at right angles to follow a parallel, upward course. Hence the characteristic "gridiron" pattern seen in Fig. 41. In the upper section, which in this type forms a triangular cap or lid, only the four carpel midribs persist. This is the case also in the siliqua type, in which the two sections are of about equal length and the veins of the lower section fewer and less prominent. In both types the solitary ovule is borne in the lower section. It is possible that the "two-storeyed" condition is the outcome of this extreme reduction in ovule formation.

With respect to the dimorphic character of the fruits it may be said that we are already familiar with instances in other genera where the gynoeceum of early flowers frequently shows a deviation from the type form significant of the phylogenetic history. These variations, it is true, are generally in the direction of an increase in carpel number (many Cruciferae, some of the Gramineae), nevertheless it seems scarcely doubtful that in the present case the sudden alteration in the dimensional relations of the two pairs of carpels, which results in the abrupt change from the urceolate to the siliquoid form, is similarly of phylogenetic significance and points to an ancestral condition in which the urn-shaped fruit was typical; or in other words to a phylogenetic history in which a fumarioid type of ovary preceded the present-day siliquoid type.

Although a consideration of the interpretation of the androeceum lies wholly outside the scope of our present enquiry, the unique distribution of the stamens of the Fumariaceae in two groups, each composed of one central whole stamen flanked on either side by a half stamen, has so great an interest in the present connection as to justify a brief digression into this field. For this  $\frac{1}{2} : 1 : \frac{1}{2}$  grouping affords a striking parallel with the  $\frac{1}{2} : 1 : \frac{1}{2}$  carpel combinations forming the unit components of the gynoeceum in certain genera belonging to other families, as e.g. Rosaceae and Liliaceae among others, a parallelism which is fully borne out by the similar mode of development in the two cases. Each  $\frac{1}{2} : 1 : \frac{1}{2}$  stamen group, according to Payer<sup>1</sup>, arises as a single protuberance. This fact is quite in accord with what is to be observed in carpel development in such cases as those instanced above. That these staminal triplets are not the outcome of the branching of two lateral stamens is shown by the mode of origin of their vascular bundles. As the cords for the two outer lateral petals leave the central vascular ring they carry out

<sup>1</sup> *Organogénie*, 1, p. 228.

conjoined with them the cords for the two lateral whole stamens (Fig. 44). The two cords originally passing out from the centre in the median plane are also composite and shortly divide to furnish the bundle for the small scale-like sepal and the superposed inner petal (Fig. 46). These are followed immediately by four very small bundles situated one on each side of both cords and destined for the four half stamens (Fig. 47). The petal tissue blocks the way to the union and consolidation of each pair of these small bundles into a single median cord. This congenital separation into two portions of the vascular component of both median stamens persists in the later development, hence the triplet arrangement. This further confirmation of the ordinary explanation of the appearance of the adult androecium of the Fumariaceae might have seemed superfluous were it not that some writers still treat this question as though the above interpretation were open to doubt.

#### CRUCIFERAE

I have already dealt in an earlier communication with the construction of the gynoeceum of the Cruciferae in general<sup>1</sup>, and in particular with that of *Capsella Bursa-pastoris* Moench. and its derivatives *C. Heegeri* Solms and *C. Viguieri* Blar.<sup>2</sup> At that time however the true nature of the character which distinguishes *C. Viguieri* was not wholly appreciated. From the further account here given it will be seen that an added interest attaches to this form in the light of the new facts.

*Capsella Viguieri* Blar. (Figs. 49-59). According to Blaringhem, who thus named a solitary individual found by him growing wild in 1908, *C. Viguieri* is primarily to be distinguished from the type form, *C. Bursa-pastoris*, by its 4-valved fruit. Individuals also show varying and often extreme degrees of fasciation of the axes. Examination of even a small population of this form renders it clear, however, that neither of these characters is altogether constant; for 3-valved fruits frequently occur and even 2-valved ones are not uncommon. [The 6-, 7- and even 8-valved specimens also occasionally met with in early flowers, as Blaringhem notes, arise from the union of two ovaries.] The fact that some *Viguieri* fruits are only 2-valved has more interest than that of a mere numerical variation, for Marchal<sup>3</sup> gives the number of chromosomes in the 2-valved type

<sup>1</sup> *Annals of Botany*, **37**, p. 460. 1923; **39**, p. 135. 1925.

<sup>2</sup> *Op. cit.* **39**, pp. 138-41 and 7 figures (p. 136).

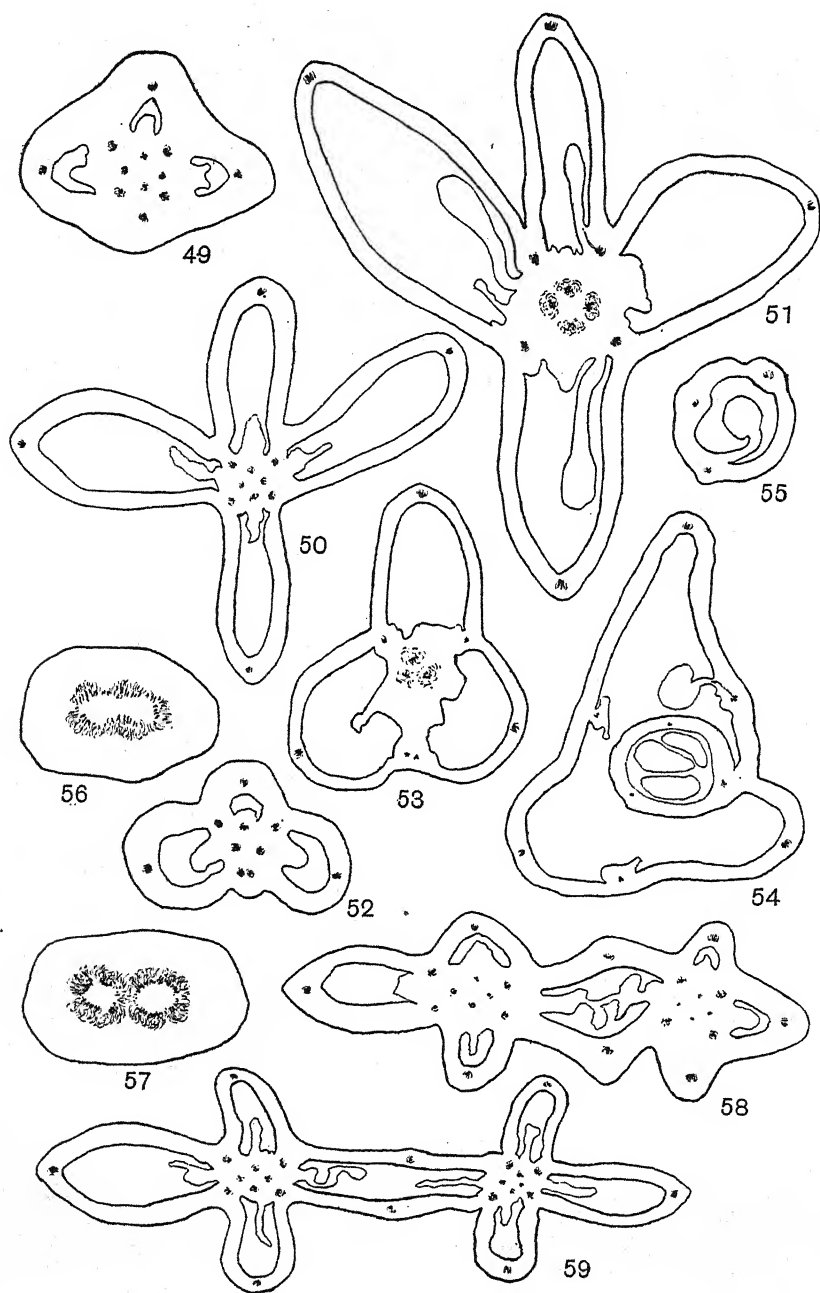
<sup>3</sup> *Mém. Acad. Roy. Belg. Cl. Sci.* **4**, pp. 8 and 22.

form, *Bursa-pastoris*, as 16 and in *Viguieri* as 8. It is not proposed, however, to pursue this point further here (material for a full investigation of the chromosome problem involved has been placed in more competent hands), but to confine our attention to the special character of the carpel scheme. From microscopical investigation it becomes apparent that the really constant feature distinguishing *C. Viguieri* is neither the precise number of the fruit valves nor the occurrence of fasciation, both of which characters, as stated above, are variable, *but the fact that the formation of two carpel whorls, one of sterile valves alternating with one composed of fertile solid members, does not use up the whole of the vascular tissue. A sufficient amount remains over to permit of the formation of a third carpel whorl.* The members of this third whorl lie on the same radii as the valve members of the first whorl, that is to say in the orthogonal planes, and alternate with the solid members of the second whorl which lie in the diagonal planes. The unusual character of the members of this third whorl is evidently the result of the peculiar environmental relations consequent upon their position. The outer epidermis and mesophyll of these innermost carpels form irregular protrusions into the loculi enclosed by the outer valve carpels, this being the only direction in which expansion is possible. Their vascular cords abut on the pith which after a while comes to an end, giving place to a cavity in which ovules may be developed by one or more of the members of this third whorl. The three whorls are isomerous and usually tetramerous but most individuals produce some flowers in which the

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Figs. 49-59. *Capsella Viguieri* Blar. All from transverse sections. 49-51. From a typical gynoeceum (whorls tetramerous). 49. Ovary base at the level of origin of the loculi of which three are already in being. The vascular cords of the three carpel whorls are clearly defined. 50. The same at a higher level showing the protrusions of the tissues of the third carpel whorl into the loculi formed by the outermost whorl. 51. The same at a still higher level. In the loculi the developing ovules arise on either side of the above-mentioned protrusions. 52-55. From a trimerous gynoeceum. The stages represented in 52 and 53 correspond with those shown in 49 and 50 respectively. 54. The same at a higher level. The carpels of the third whorl have formed a second ovary within the first, and have produced ovules in the locus which appears as the pith comes to an end. The ovules in the original ovary are borne as usual by the solid members of the second whorl. 55. The same inner ovary at a higher level, now disjoined from the wall of the outer ovary and standing free in the locus. 56-59. From a 6-winged gynoeceum. 56. The ovary base with single vascular cylinder. 57. The same from a higher level showing bifurcation of the vascular cylinder. 58. The resulting double ovary composed of two sets of three tetramerous whorls so disposed as to render the whole gynoeceum bilaterally symmetrical. 59. The same at a higher level.





gynoecium is composed of trimerous or occasionally even of dimerous whorls. Examination of 6-, 7- and 8-winged ovaries shows that they arise from "twinning" consequent upon bifurcation of the central vascular cylinder after the giving off of the stamen cords, and before differentiation of the carpels. In the fully formed double ovary two opposite valve carpels extend between and serve to connect the two 3-valved portions which make up the rest of the structure. According as these two connecting valve carpels are both flattened (with linear cross-section), or one is flattened and one folded (with V-shaped cross-section), or both are folded, the gynoecium appears as a 6-, 7- or 8-winged structure. As noted in an earlier account<sup>1</sup>, 5- and 6-valved fruits also occur occasionally in *Tetrapoma*. These instances were observed in herbarium material, and the question whether bifurcation of the vascular cylinder accounted for the extra carpels here too could not be determined. But the fact that these exceptional *Tetrapoma* fruits appeared to exhibit strict radial symmetry does not support this interpretation, in view of the marked bilateral symmetry characterising those fruits of *C. Viguieri* in which bifurcation was observed (see Figs. 58, 59).

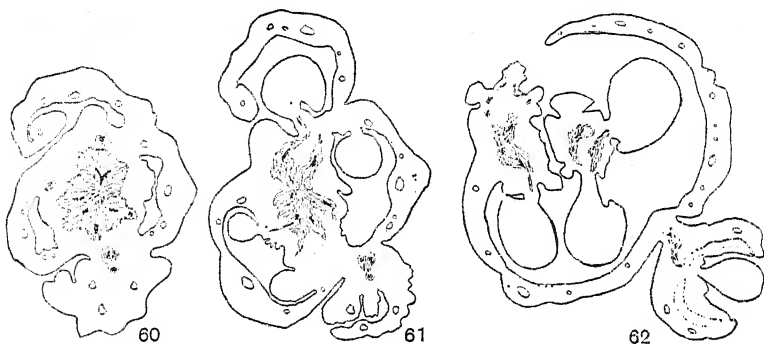
#### CARYOPHYLLACEAE

*Saponaria officinalis* L. The normal gynoecium of *Saponaria*, as shown already for *Dianthus*<sup>2</sup>, is composed of two median, sterile, valve carpels, each surmounted by a style, and two lateral, fertile, solid, styleless carpels. A semi-double form of this species is of considerable interest in the present connection for many of the flowers show an increase in the number of carpels accompanied by various malformations of the pistil such as partial disjunction of carpel groups with resultant production of ovules on the margins of the valves as well as on the solid carpels. The largest number of carpels observed in these flowers was eight. A case of this kind, where the gynoecium consisted apparently of two tetramerous whorls, the outer of valve, the inner of solid carpels, is shown in Figs. 60-62. In the basal region of this gynoecium the whole vascular system destined for the inner carpel whorl was consolidated into a single irregular central mass, but at higher levels the partial disjunction of some of the carpels allowed the general ground plan to become apparent. A couple of carpels, one valve and one solid, became separated off on the one side and together formed a small, distinct

<sup>1</sup> *Annals of Botany*, 37, p. 464.

<sup>2</sup> See Carpel Polymorphism, I, *Annals of Botany*, 39, p. 142, and Figs. 36, 37, 1925.

ovary. A similar pair behaved in like manner on the other side, leaving the central ovary now fairly normal in appearance and showing plainly its two valve and two solid members. Five styles were present, one being borne by each of the four proper valves and one by the second member of one of the two small dimerous ovaries, *this member having undergone a change in character from the consolidated form below to valve form above*. This fact alone is sufficient proof of the true carpel nature of this second member, but as is so often the case with aberrant forms other fresh and convincing evidence of carpel polymorphism was furnished by this specimen<sup>1</sup>.



Figs. 60-62. *Saponaria officinalis*, semi-double variety. All from transverse sections taken at successively higher levels of a gynoecium of eight carpels. 60, 61. The two valve carpels to right and left already enclose each a loculus. Below, a valve and a solid carpel are about to separate from the rest of the gynoecium and to form a small, separate, dimerous ovary. Above, the remaining valve carpel is already partly free from the main body of the gynoecium and has developed an ovule on the disjoined margin. The solid carpels of the inner whorl are still in process of differentiation. 62. One of the two small, dimerous ovaries has become completely disjoined and is no longer seen, the other will also shortly be completely free. The two solid carpels of the central ovary, now fully differentiated, have separated from each other, the one on the left giving a false appearance of parietal placentation (as formerly understood).

It was long ago noted by Masters<sup>2</sup> that both marginal and free central (so-called) placentation were often to be met with in the double form of *Saponaria*. On the old monomorphic view such a combination should be a morphological impossibility since a fertile carpel margin cannot be supposed both to remain the actual edge of the carpel lamina, and at the same time to be separated off and

<sup>1</sup> See e.g. the case of *Cheiranthus Cheiri* var. *gynantherus*, *New Phytologist*, 27, p. 50, and Figs. 22-27. 1928.

<sup>2</sup> *Vegetable Teratology*, p. 97. 1869. Also *Journ. Linn. Soc.* p. 1. 1867.

form a part of the central vascular column, as this view would require in these cases. On the theory of carpel polymorphism, on the other hand, there is no fundamental difficulty in a valve member, ordinarily sterile when conjoined with fertile, solid members, itself becoming fertile along its free margins on becoming disjoined from these members. A somewhat comparable case has already been described in the case of the single and double forms of *Kerria japonica*<sup>1</sup>.

In concluding I wish to express my very grateful thanks to Miss D. F. M. Pertz for making the drawings here reproduced.

<sup>1</sup> *Annals of Botany*, 41, p. 604 and Fig. 198. 1927.

# NOTE ON THE PRESENCE OF MYCORRHIZA IN THE ROOTS OF SALT MARSH PLANTS

By EDNA MASON, M.Sc.

(With 3 figures in the text.)

MODERN research has shown that mycorrhizal fungi exist in a very large number of higher plants. Outstanding examples of mycorrhizal plants are conifers, "Amentiferae," orchids and members of the Ericaceae. According to Dr Rayner<sup>(5)</sup> mycorrhizal fungi are most abundant in plants growing in woods on forest mould and are

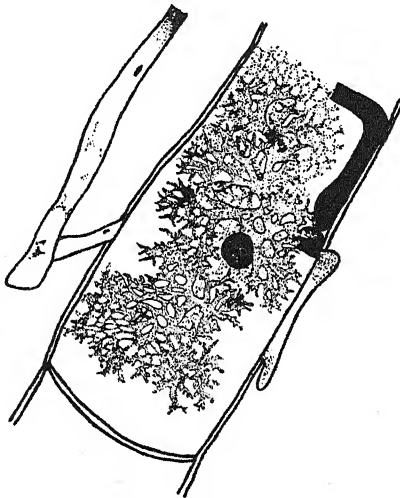


Fig. 1. "Arbuscule" in a cortical cell of a fine root of *Armeria maritima*.  
( $\times 750$ .) Note the cell nucleus.

rare in water and wet soils, except in the case of bogs. No mention is made of the salt marsh.

The object of the present note is to draw attention to the presence of endotrophic mycorrhiza, which, as far as I know, has not been previously recorded, in the roots of some of the halophytes growing in the wet salt soil of salt marshes at Borth and Talsanau. The species which possess mycorrhiza are: *Plantago coronopus*, *P. mari-*

*tima*, *Aster tripolium*, *Glaux maritima*, *Armeria maritima*, *Cochlearia officinalis*, *Agrostis alba* and *Glyceria maritima*.

When examining longitudinal and transverse hand sections of the very fine rootlets of the above species definite fungus hyphae were revealed in the cortical cells and in most cases the mycelium consisted of branched systems of unseptate hyphae, varying from 2 to  $9\mu$  or more in width and being sometimes lobed and of irregular contour. They did not appear to cause any hypertrophy, but simply ramified through the cells. At intervals they generally produced the characteristic compound "arbuscules" (Fig. 1) similar to those

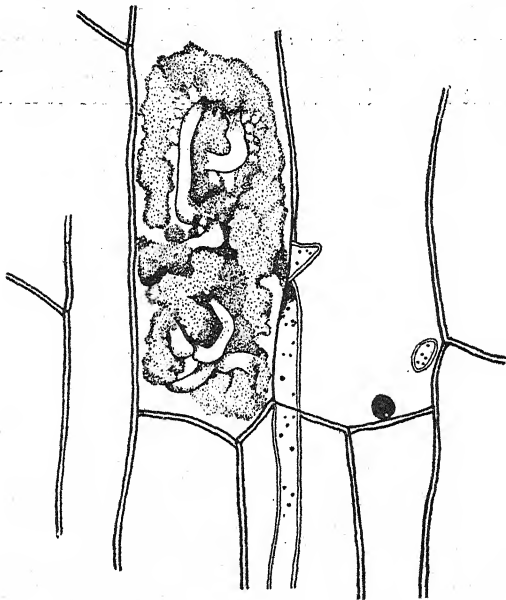


Fig. 2. "Sporangiole" in a cortical cell of a fine root of *Armeria maritima*. ( $\times 750$ .)

figured by Gallaud(2) and Janse(3). These were very evident in *Plantago* spp., *Armeria maritima* and *Glaux maritima*, where, under very high magnification, fine networks of branching filaments are seen.

"Arbuscules" were found in various stages of intracellular digestion, the resulting granular masses being equivalent to the "sporangioles" spoken of by Janse(3) (Fig. 2).

This Phycomycete type of mycelium was very abundant in the roots of all the halophytes mentioned. As yet, no mycorrhizal

invasion has been seen in old roots, rhizomes, aerial stems and leaves of these halophytes nor in the following species: *Salicornia europaea*, *Spergularia marginata*, *Triglochin maritimum*, *Juncus maritimus* and *J. gerardi*.

Vesicles were found on unseptate hyphae, similar to those figured by Peyronel (4) in wheat. In material collected in July and August they showed vacuolated cytoplasm and many nuclei, but none have been found in the spore stage.

In *Glyceria maritima* the mycelium showed dimorphism—hyphae of non-septate and also of septate type. The septate hyphae branched and were intracellular, but so far have not been seen in typical "pelotons" (Fig. 3). The occurrence of the two types of mycelium supports Peyronel's theory (4) of "double infection." Septate hyphae have also been observed in roots of *Armeria maritima* and *Agrostis alba*.

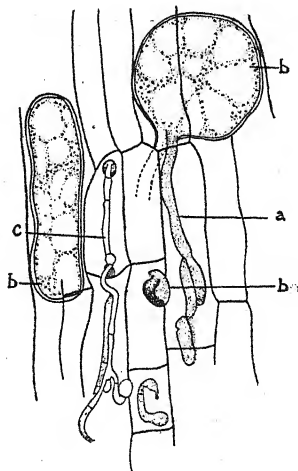


Fig. 3. L.S. fine root of *Glyceria maritima*. a, unseptate hypha; b, vesicle; c, septate hypha. ( $\times 240$ .)

My thanks are due to Dr Bayliss Elliott and Dr Rayner for examining my preparations and confirming my observations.

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## REVIEW

*The Structure and Development of the Fungi.* By H. C. I. GWYNNE-VAUGHAN and B. BARNES. Demy 8vo. Pp. xiv + 384, with frontispiece and 285 text-figures. Cambridge University Press, 1927. 15s. net.

Lecturers on Mycology have long wanted an English text-book which could be recommended to advanced students. The present volume fulfils this need most admirably. The senior author has already written a book on certain groups of fungi, but "*The Structure and Development of the Fungi*" covers the whole range of these organisms, and deals more fully with their physiology and general biology than does the earlier book. In addition, this volume contains a very valuable chapter on mycological technique, which includes much useful information on the preparation of culture media and the use of stains. The references to literature are very full, and are included in a bibliography at the end of the book.

The classification of these organisms which has been adopted is lucid and logical. The simplest fungi are included in the Archimycetes, a group co-equal with the Oomycetes. Among the Ascomycetes the group of the Plectomycetes, instituted first by the senior author in her earlier work, is advantageously retained. In regard to minor matters the reviewer is somewhat surprised at the inclusion of the Protomycetales in the Archimycetes, and it would seem that the time has come to break the convention of placing the Uredinales in close juxtaposition with the Auriculariales.

The book is very profusely illustrated, and the drawings are beautifully clear. Apart from the authors' own splendid figures mycological literature appears to have been thoroughly ransacked for the selection of a truly wonderful series of illustrations. The letter-press is fully up to the high standard of the Cambridge University Press. Misprints are conspicuous by their absence, but on page 336, in the formulae for bleaching agents, potassium chloride should be potassium chlorate.

The information given in the book is very clearly expressed and is presented without bias. The standard of accuracy is extremely high. The reference on page 14 to *Sphaerotilus natans*, however, implies that this organism is a fungus, although most authorities consider it to be a filamentous bacterium, notwithstanding its popular name of "sewage fungus."

Both teachers and students of Mycology will warmly welcome the appearance of this book, and the authors are to be heartily congratulated on the service they have rendered to the advancement of our knowledge of the fungi.

F. T. BROOKS.



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## ILLUSTRATIONS OF CARPEL POLYMORPHISM III

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(With Plate IV and 53 figures in the text.)

EVIDENCE was adduced in an earlier account<sup>1</sup> showing that the full ground plan of the gynoecium in Geraniaceae, Linaceae, Oxalidaceae and Tropaeolaceae consists of *two* whorls of five carpels each<sup>2</sup>. In support of this interpretation descriptions or figures were given of certain geraniaceous types (*Geranium*, *Erodium*, *Pelargonium*), of *Linum* and *Tropaeolum*, in which last-named genus, however, some of the carpels are habitually suppressed. It is proposed in the present account to deal with *Limnanthes*, formerly included in the Geraniaceae, now transferred to a distinct family—the Limnanthaceae, with the Oxalidaceae in greater detail, and with the Balsaminaceae, since all these forms differ in certain important respects as regards the gynoecium from the types already considered. Figures are also added of *Tropaeolum* showing stages in degeneration of the vascular cords which correspond to the missing carpels.

### LIMNANTHACEAE

*Limnanthes Douglasii* R.Br. (Figs. 1-3). Here, as in the Geraniaceae, the gynoecium consists of two pentamerous whorls, the one composed of valve, the other of solid carpels, *but the rôles of the two whorls are reversed in the two cases*. For in *Limnanthes* it is the antepetalous members which bulge out and enclose the loculi, and the antepetalous carpels which remain central and are prolonged upwards

<sup>1</sup> "Carpel Polymorphism I," *Ann. Bot.* 39, pp. 147-151, 1925.

<sup>2</sup> On the former monomorphic view only one pentamerous whorl is supposed to be present.

to form the (so-called gynobasic) style column. Consequently the flower of *Limnanthes* is diplostemonous, whereas those of (? all) geraniaceous genera are obdiplostemonous, as is natural where the antesealous carpels are solid and remain central and the antepetalous members bulge out in order to form a loculus.

Almost the whole xylem component of the cord running to the fertile antesealous valve carpels of *Limnanthes* branches off to supply the solitary basal ovule. The midrib portion is thereafter reduced to a fine, undifferentiated strand which only persists for a very short distance, coming to an end soon after the level of the loculus floor is reached (Fig. 1). The outer wall of the chambered region of the ovary is thus devoid of vascular tissue. Owing to the separation of these carpels the ovary soon becomes partially apocarpous (Fig. 2). The effect is to make the style column, which is formed of the five conjoined and consolidated antepetalous carpels, appear "gynobasic"; in reality the column is the direct prolongation of these members of the inner whorl (Fig. 2). Each individual component style contains a single vascular strand—the midrib cord of the carpel—and becoming free towards the apex terminates in an undivided stigma standing over the petals.

#### OXALIDACEAE

In *Oxalis*, by far the largest genus in the Oxalidaceae, the outstanding feature distinguishing all the species so far investigated is the marked degeneration of the carpel whorl standing in line with petals and loculi. In these carpels, which are sterile, the xylem elements are either reduced to a minimum, or more often are lacking altogether. Further, with the disappearance of the xylem the midrib bundles of these carpels fail in many species to connect below with the general vascular system. They come into being free and unattached, appearing as undifferentiated strands in the wall of the ovary at the outer border of each locus. Precisely the same process of degeneration is, in fact, in progress here as has been described for the valve carpels of many of the Papaveraceae<sup>1</sup>. These downgrade changes in the antepetalous carpels have led in some species to a compensating development in the antesealous fertile members. The nature of this change of balance between the two carpel whorls and the extent of the degeneration process will be most readily appreciated if we deal first with the small genus *Averrhoa*, which has

<sup>1</sup> See "Illustrations of Carpel Polymorphism I," *New Phyt.* 27, p. 52, 1928.

not yet suffered marked deterioration in carpel development and hence shows the original ground plan in its fullest development.

*Averrhoa Bilimbi* L., *A. Carambola* L. Transverse sections through the flower base show that the antepetalous carpel midribs leave the residual vascular ring as strongly developed strands well supplied with xylem. They, with their xylem components, are prolonged to the top of the style filaments which stand in line with the petals. These carpels are of the valve type, the midribs giving rise to one or two lateral veins on each side at the base, but, unlike the valve carpels of the Geraniaceae, they are sterile. The antesepalous carpels are solid and fertile. Their vascular cords, which only take shape at a higher level<sup>1</sup> (see Fig. 40) remain central. At the ovary apex these cords divide in two, one-half running to the style filament over the valve on the right, the other to the valve filament on the left. Hence each filament contains three distinct vascular strands corresponding to the  $\frac{1}{2} \times 1 \frac{1}{2}$  carpel construction which is characteristic of this genus as of various genera in certain other families (e.g. Rosaceae, Liliaceae). In *A. Carambola* this construction is reflected in the slightly trefoiled outline of the individual stigma.

As stated above, carpel development reaches its highest level in *Averrhoa*, a level from which all the species of *Oxalis* so far investigated depart in a greater or less degree, as will be seen from the following account, in which the species examined are grouped according to the amount of degeneration which has taken place.

*Oxalis scandens* H. B. and K., ls<sup>2</sup> (Figs. 4-14), *O. corniculata* var. *atropurpurea* Planch., ms (Figs. 15-19), *O. corniculata* type ms, *O. chrysantha* Prog. ls.

A cross-section through the flower of *O. scandens*, a climbing species, taken immediately above the emergence of the stamen cords, shows the residual vascular tissue in the form of a pentagon consisting of ten xylem-containing bundles of which five, with a mere xylem remnant, constitute the angles of the pentagon and stand in line with the antepetalous stamens, now already beginning to take up a position as the outer whorl (Fig. 7). The other five bundles,

<sup>1</sup> The reformation of a ring after the emergence of the antepetalous carpel midribs, before the residual vascular elements are reconstituted into the cords of the antesepalous carpels is one of the countless minor structural features irreconcilable with the old idea that these latter cords represent conjoined marginal veins belonging to the antepetalous carpels.

<sup>2</sup> Long-styled (ls), mid-styled (ms) and short-styled (ss) forms behave alike, the style length is cited in each case merely for completeness.

containing several xylem vessels and standing on the alternate radii form the sides of the pentagon figure, the centre of which is occupied by pith. The whole of each antepetalous bundle shortly runs out horizontally towards the periphery (Fig. 8), leaving a broad medullary ray in its place and giving off almost immediately a pair of lateral veins (Fig. 9). These lateral veins appropriate the whole of the xylem component. As the locus makes its appearance between them and the midrib curves upwards at its outer border, the latter bundle no longer shows any vessels but continues thenceforward as a strand of unlignified elements (Figs. 10-13) which are traceable as far as, and even into, the style filaments, though the two lateral veins cease at the ovary apex (Fig. 11). The antepetalous carpels are thus, as in *Averrhoa*, of the valve type, and here also these carpels are sterile. The antesepalous carpels, on the other hand, are consolidated and fertile. Their midribs continue upwards in their central position, giving off a placental branch towards the apex (Fig. 11) which supplies the usually solitary ovule in each locus. It was observed incidentally that the ovules were not regularly borne, one by each member of the whorl. One carpel might be vigorous enough to produce two ovules, one in the locus on the right and one in that to the left, another might be too weak to bear even a single ovule, while others might produce one on the one side but not on the other. It is obvious that when reduction has reached this point the further downgrade step to a trimerous condition in which each solid member has room for full fertility would not be any detriment to the plant and would have the advantage of raising the ovule output from five to six.

As the ovary is about to contract into the style column the pith disappears, the consolidated carpels are left free at their inner boundaries, and the ovary for a short distance becomes unilocular (Fig. 12). At about this level these solid carpel midribs divide radially, the two halves passing outward and thence upward, the one into the style filament centred over the valve carpel on the right, the other into the corresponding style filament on the left, consequently each filament shows two xylem-containing strands, each corresponding to a half carpel (Fig. 14). These bundles continue to the top and account for the hitherto unexplained bifurcate form of the stigma characterising the whole genus, for though the undifferentiated strand of the valve carpel midrib may be continued into the filament, it is too weak to affect the outward form. Each style filament is thus, as in *Averrhoa*, a compound structure having the

composition  $\frac{1}{2} + \frac{1}{2}$  carpels, though the one whole carpel no longer shows differentiated vascular tissue at this level<sup>1</sup>.

Mention has been made above of slight differences in vigour among the five consolidated members resulting in differential fertility, in cases where space permits of the formation of only five ovules, though the number of potential points of origin is ten. A reflection of this inequality may sometimes be seen in a style filament, one of the half carpel cords showing a continuous thread of one or more spiral vessels, while in the other the vessels may cease altogether for a longer or shorter distance and reappear at a higher level (Fig. 14), a condition which recalls a similar discontinuous differentiation in the case of the solid member of the much-reduced gynoeceum of *Alchemilla*<sup>2</sup>.

*O. corniculata* var. *atropurpurea* Planch. (Figs. 15-19) and *O. chrysantha* Prog. show substantially the same features as those described above for *O. scandens*, except that here the ovules are borne in two rows in the loculi. In *O. corniculata* type, however, the vascular development seems rarely to reach this level. In the material examined it was the exception rather than the rule for the antepetalous cords to turn out from the central cylinder and to form the characteristic pair of lateral veins. Even when such was the case no xylem vessels could be detected, either in these laterals or in the cord itself.

The later secondary veins which develop in the ovary wall in the fruiting stage (traced only in the case of *atropurpurea*) are derived from the *sterile valve carpel vascular system*. They, naturally, have an *upward* trend, and may show an occasional anastomosis with the fertile carpel cords.

The level of development of the epipetalous carpel vascular system reached in the three species cited above is exceptional, and represents high-water mark for the thirty-four species examined, and possibly for the whole genus.

*O. stricta* L., ms (Figs. 25, 26), *O. lasiandra* Zucc., ss, *O. Martiana* Zucc., ms. In these species, as in the preceding group, the antepetalous carpel cords turn out from the central cylinder and develop xylem vessels. But in *O. stricta* and *O. lasiandra* the cords do not

<sup>1</sup> In two or three species included in later groups (*O. incarnata* L., *O. Origiesi* Regel, *O. tuberosa* Molina) the differentiation in this strand of a single spiral vessel, extending for a longer or shorter distance, was occasionally observed.

<sup>2</sup> See "Carpel Polymorphism II," *Ann. Bot.* 41, p. 575, and Fig. 20, p. 572, 1927.

branch at once, but run out horizontally for some distance before giving rise to two lateral veins which, however, very soon cease. These veins contain no xylem, and that of the main bundle also here comes to an end, so that a differentiated midrib bundle is not traceable beyond this point. In *O. Martiana* the cord forms no laterals, and consequently retains its xylem component for a little distance up the outer wall of the loculus. In the flowering stage the loculus wall is without secondary veins, but as the fruit develops the side walls become supplied with a few lateral veins derived from the *fertile consolidated carpels* (*O. stricta*, *O. lasiandra*)<sup>1</sup>. These veins take their rise in the upper portion of the ovary and have a *downward* trend.

Here we see retrogression in the vascular development of the antepetalous carpels met by a compensating further development in the antesealous members which, however, in the above forms is not initiated until after fertilisation. (In the larger group dealt with below this response is thrown back to an earlier stage, being manifested in the flowering condition.) In other words, as the antepetalous carpels lose their valve character and contract to the solid form the consolidated episepalous carpels expand and assume the semi-solid character.

An unidentified, long-styled, pink-flowered form from the Andes may be regarded as illustrating the next grade in the scale. In this form antepetalous midrib bundles which turn out have all but vanished altogether. Only sometimes, on one or perhaps two radii, is a very small undifferentiated antepetalous strand seen to get so far as just to leave the central cylinder. But these strands could be traced no further, and no corresponding ones would be observed on the other three or four radii, all the remaining residual vascular elements being consolidated into the antesealous carpel cords. We appear here to catch a last glimpse of the connecting basal end of these antepetalous midribs before this portion is suppressed completely in the whole whorl. This reduction process is here further marked by the presence in the pith at this level of a few xylem elements, a feature otherwise only observed in *O. Acetosella*, where also we catch a last view of vessels which, no longer utilised, make their way into the pith and are discarded (see later, p. 207).

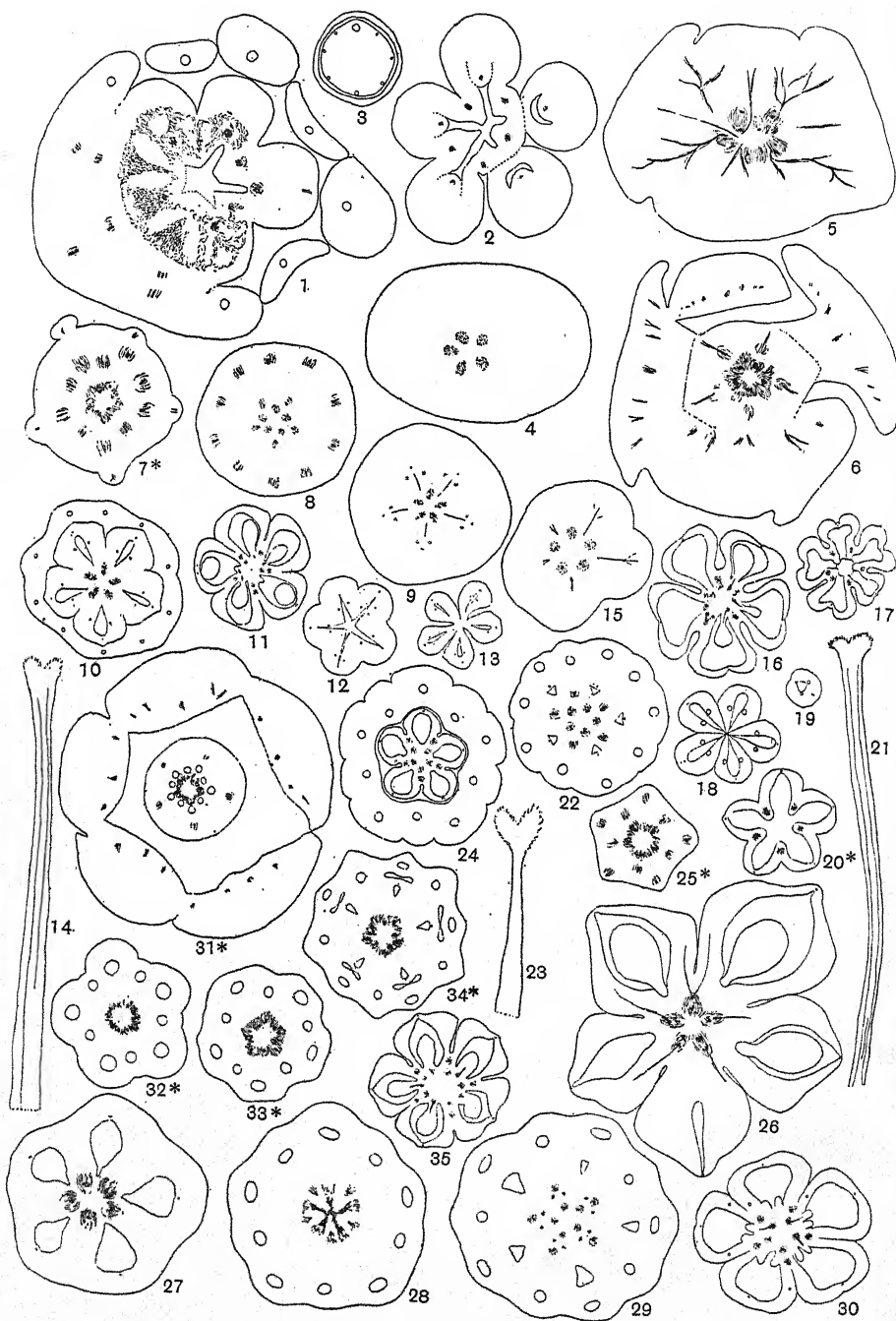
In the great majority of the species examined, however, a further stage in the downgrade process has been reached, the lowest con-

<sup>1</sup> No material of *O. Martiana* in the fruiting stage was available for comparison.

necting portion of the antepetalous midribs being no longer traceable. There is no turning out horizontally from the central cylinder just below the loculus level of five xylem-containing bundles on these radii. Only as the loculi actually come into being are the peripherally situated, undifferentiated midrib-strands seen suddenly to appear in the ovary wall, their lower ends thus being unconnected with the rest of the vascular system.

In such species, e.g. *O. articulata* Savign., ms, *O. hirta*\* L., ls, *O. adenophylla*\* Gill., ms, *O. bupleurifolia*\* A. St Hil., ss, *O. carnos\** Molina, ms (Fig. 36), *O. cernua*\* Thunb., ss, *O. Deppei*\* Lodd., ss, *O. enneaphylla*\* Cav., ms (Figs. 28-30), *O. floribunda* Lehm., ms, *O. gigantea*\* Barn., ls, *O. incarnata* L., ls (Figs. 20, 21), *O. filicaulis* Jacq., ms, *O. latifolia* H. B. and K., ss, *O. lobata*\* Sims, ms (Figs. 22, 23), *O. purpurata*\* Jacq., ss, *O. purpurea* L., ms, *O. tuberosa* Molina, ss, the residual vascular tissue shows ten xylem-containing cords arranged, as in the preceding cases, in pentagon form, but here it is the five in line with the sepals which stand further from the centre and form the angles of the figure. The inner alternate bundles or bundle masses at the sides of the pentagon represent, not whole antepetalous cords, but placental strands which no longer connect with their corresponding midribs owing to the complete degeneration of the basal region of these latter bundles, which now only make their first appearance at the outer border of the loculus. These antepetalous placental strands soon divide into two halves, each half becoming merged in the placental strand of the neighbouring antesepalous carpel to right and left respectively, from which, as in the preceding group, the branch to the funicles is derived. The style filaments show the usual two vascular strands corresponding to one half of the antesepalous carpel to right and left. But in the middle region of one style filament in a flower of *O. incarnata* a single spiral vessel could be traced for some distance along the otherwise undifferentiated antepetalous carpel strand (Fig. 21), and in a flower of *O. tuberosa* one filament showed such a vessel running almost throughout the entire length of the strand.

In the species marked by an asterisk secondary veins are already present in the walls bounding the loculi in the flower, in others (e.g. *latifolia*) they do not make their appearance until the fruiting stage. In both cases, as we are prepared to find from what has gone before, these veins originate from the antesepalous carpels and pursue a downward course. Regarding other species lacking these veins in the flowering stage but of which no material in fruit was



Figs. 1-35.

Figures marked with an asterisk (7, 20, 25, 31, 32, 33, 34) are reproduced on an enlarged scale in Plate IV at the end of the paper.



Figs. 1-35. All from transverse sections taken at successively higher levels except 14, 21, 23. 1-3. *Limnanthes Douglasii* R.Br. 1. The flower base, one side of which is more developed than the other. On the right the antesepalous stamens with accompanying glands are pushed out by the bulging antesepalous valve carpels. One of these carpels (on the right) shows the midrib reduced to a fine undifferentiated strand after having given off a strong placentated branch. The cords of the inner solid carpels are seen taking shape on the alternate radii. On the left the boundaries of perianth, androecium and gynoecium are not yet defined though the cords for the members of the four outer whorls are in being. Those destined for the carpels are still in process of differentiation. In the centre the pith is already giving place to a common style canal. 2. The gynoecium. The valve carpels are becoming disjoined from each other and from the inner carpal whorl which is continued upwards as the (so-called gynobasic) style column. The loculi and developing ovules are becoming defined. In the three carpels on the left the loculi are seen to be continuous with the stylar canal. 3. A single valve carpal with ovule. The ovular integument shows a strongly developed vascular system. 4-35. *Oxalis* spp. 4-14. *O. scandens* H. B. and K. 4. The flower stalk. 5. The flower base at the level of origin of the venation system of the calyx (see p. 208). The strands which become the sepal midribs take their rise from the inner face of the residual vascular masses and bend horizontally outwards. Branches arising from almost the same point turn outwards round either side of the same mass and form the venation system along the line of junction of contiguous sepals. 6. The same at the level of origin of the petal cords which arise in the ordinary way. 7. The same after the emergence of the ten stamen cords. The residual vascular tissue forms a pentagon figure of ten bundles enclosing pith. 8. The same after the five bundles at the angles of the pentagon have turned out from the centre to form the antepetalous carpal midribs, leaving behind in their central position the five alternate bundles which become the cords of the antesepalous carpels. 9. The same after the antepetalous carpal cords have given rise to a lateral vein with xylem, on each side of the developing loculus, the midrib itself being thereafter reduced to an undifferentiated strand. The antesepalous carpal cords show already two xylem strands destined for the midrib and placental branch (both with xylem). 10. The ovary surrounded by, but now free from, the androecium tube, with the loculi well developed. 11. The same showing the ovules supplied by one of the placental bundles of the solid antesepalous carpels. In the valve carpels only the midrib strand now persists. 12. The ovary apex. The valve midrib strands may still be traced. The fertile carpal cords have divided in half, the halves separating and passing outwards into the lateral wall of the loculus. 13. The style column. The vascular system as in 12. 14. A single style filament rendered transparent and mounted whole. The half cord of the one component solid carpal showed a continuous strand of xylem vessels up to the stigma; in the other half cord lignification was discontinuous, vessels being absent from the basal portion. 15-19. *O. corniculata* var. *atro-purpurea* Planch. 15. The ovary base. The five antepetalous carpal cords have left the centre and are forming the pairs of lateral veins. The antesepalous cords remain central. 16. The ovary at the level of attachment of the ovules which are served by the placental branch of a solid carpal. 17. The ovary just before it becomes unilocular owing to the disappearance of the last remnant of pith. One of the solid carpal midribs has divided in two. 18. The ovary apex. The half cords of the split solid carpels have moved outwards into the lateral walls. 19. A single style filament with the three strands representing  $\frac{1}{2}$   $\times$   $\frac{1}{2}$  carpels disposed to one side of the stylar canal. 20, 21. *O. incarnata* L. 20. The ovary now one-chambered owing to the disappearance of the pith. The xylem strand in the antesepalous carpal midribs has divided in two preparatory to the complete halving of the midribs. 21. A single style filament showing a short strand of xylem vessels due to intercalary lignification in a third vascular strand (= the antepetalous carpal midrib) in addition to the two half cords of the neighbouring antesepalous carpels. 22, 23. *O. lobata* Sims. 22. The conjoined androecium and gynoecium at the level of origin of the loculi showing the midribs (at the outer border of the loculi) and placental bundles of the antepetalous carpels and the trunk cords of the antesepalous whorl. 23. Upper portion of a single style filament with conspicuously bilobed stigma. (The

available (*articulata*, *floribunda*, *incarnata*, *purpurea*, *tuberosa*, *filicaulis*), we may safely conclude that if secondary veins are formed after fertilisation they will arise from the fertile carpel vascular system.

*O. rubra* A. St Hil., ls (Fig. 24) and *O. valdiviensis* Barn., ss, *O. vespertilionis* Zucc., ss, and a form which is possibly *O. Grahamiana* Benth., ss, show the same disposition of the vascular bundles as that described above, but they stand one grade lower in the scale, for only the five antesealous cords possess xylem vessels, the xylem elements in the antepetalous placental strands having already come to an end before the reconstruction and consolidation of the antesealous carpel cords have been accomplished.

In *O. magellanica* Forst., ls (Fig. 27), *O. Acetosella* L., ms, *O. dispar* N. E. Br., ss, and *O. rosea* Sims (?), ls, we see the last stage. The central residual vascular tissue is reduced from the first moment after the emergence of the stamen bundles to the five antesealous cords. The antepetalous placental strands which in the preceding group are still to be traced, though they contain no xylem, have now

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vascular strands are not shown.) 24. *O. rubra* A. St Hil. The gynoecium surrounded by the stamen tube. The antepetalous carpels with midrib strand only, the antesealous members with midrib and placental bundles. 25, 26. *O. stricta* L. 25. The flower base showing the ten cords for the androecium and the residual vascular ring subserving the gynoecium, consisting of five xylem-containing bundles and five alternate bundles lacking xylem elements. 26. Young fruit showing the secondary venation of the valves arising from the fertile carpels. 27. *O. magellanica* Forst. The gynoecium immediately above the level or origin of the loculi. At their outer border the undifferentiated strands of the antepetalous carpel midribs which no longer connect below with the central cylinder, the whole residual vascular tissue at this lower level being consolidated into the five antesealous carpel cords (see fuller description above). 28-30. *O. enneaphylla* Cav. 28. The conjoined androecium and gynoecium below the level of origin of the loculi. The residual vascular cylinder shows five outer antesealous cords and five antepetalous large irregular bundle masses destined for the ten carpels, surrounded by the ring of ten cords for the androecium. 29. The same at the level of origin of the loculi. The original antepetalous bundle masses after dividing in two have diverged and merged with the vascular complex representing the antesealous carpel system. 30. The same showing the funicle branch arising from the placental strand of the antesealous carpels. 31-35. *O. Origiesi* Regel. 31. The flower base. The sepals are becoming free. The cords of the petals and stamens have already left the centre leaving behind a pentagon figure with ten groups of xylem, the angles being in line with the petals. 32, 33. The conjoined androecium and gynoecium. 32. The antepetalous stamen cords together with the bulging semilunar glands now lie further out than the antesealous cords. The residual vascular tissue now appears as a ring as the angles of the pentagon flatten out. 33. The pentagon figure reappears but now has the angles in line with the sepals (see explanation in the text, p. 207). 34. The androecium and gynoecium just before they become disjoined. The loculi are seen appearing between the antepetalous stamen cords and the sides of the central pentagon figure. 35. The gynoecium at the level of attachment of the ovules which are supplied by an antepetalous placental strand.

disappeared altogether. A feature observed in *O. Acetosella* may well be connected with this reduction. Notwithstanding the small size of the residual vascular ring in this species, a considerable portion of the xylem displaces the pith and is discarded.

From the above account it will be seen that we are able to trace within the genus *Oxalis* a gradual change in character of the sterile antepetalous carpels, accompanied by progressive degeneration. At the one end of the scale we find the condition in which the valve character is clearly indicated by the formation of a pair of lateral veins which run in the outer wall of the ovary (*O. scandens*, *O. corniculata* var. *atropurpurea*, *O. chrysantha*). At the other end, that in which these carpels have contracted and become consolidated; the midrib, consisting of undifferentiated elements, no longer connects below with the general vascular system, and it forms neither the sterile lateral veins of the persisting valve type nor the medianly situated but functionless placental strands seen in the intermediate forms. Between these two extremes stand the bulk of the species, in which the antepetalous carpel has become consolidated but in which, though the basal region of the midrib has disappeared, placental strands are to be found corresponding with those of the antesealous carpels and with which they soon become merged.

Furthermore this contraction and degeneration of the antepetalous carpel whorl has been met by a compensating development in the antesealous whorl. It may be that this readjustment, possibly also the retrogressive change which called it forth, is of more recent origin in some species than in others, since in some it has already taken place in the flowering stage while in others it can only be detected during the development of the fruit.

The one remaining species investigated—*O. Ortgiesi* Regel, ms (Figs. 31-35)—is deliberately treated apart from the rest, for it appears to stand in a class by itself. Like several of the forms already considered, it shows a residual ring of ten xylem-containing bundles which become disposed in pentagon form with the angles in line with the sepals. But it is unique among these forms in that the antepetalous bundles forming the sides of the pentagon, as well as the antesealous bundles at the angles, retain their individuality and their xylem component up to the ovule-bearing level. At the outer border of the loculus is the usual midrib strand of the antepetalous carpel, here, as in most of the species examined, disconnected from the rest of the vascular system and lacking xylem elements, all the xylem vessels on these radii having been retained in the placental strands

forming the direct prolongations of the antepetalous carpel cords. In all the other species investigated possessing corresponding bundles (i.e. all except those in the first group) these bundles break up at once into twin strands which become merged with the placental strand of the antesepalous carpel to right and left respectively. In *O. Ortgiesi* not only do these antepetalous placental strands persist, but, if the appearance in transverse section was correctly interpreted, it is they which serve the lowermost ovules. They then come to an end, the upper ovules being served in the usual way by the placental strands of the antesepalous carpels. Thus we appear to have in *O. Ortgiesi* a unique case in which both sets of carpels are fertile. The necessarily close arrangement of the ten placental bundles makes it less easy than in any other species to trace the course of individual strands, but that the above interpretation is correct seems fairly certain; the fact that the antepetalous carpel placental strands prove to be fertile makes their unique persistence at once intelligible. Intercalary lignification of a single spiral element was observed in the antepetalous carpel strand in the case of one style filament.

*Biophytum dormiens* G. Don (Figs. 37-39), *B. proliferum* Edgew., *B. sensitivum* D.C.

In *Biophytum* the general ground plan, which is similar to that in *Oxalis*, is exhibited in perhaps its simplest and most diagrammatic form owing to the absence of any complex rearrangement of the vascular elements after the emergence of the cords for each successive whorl. The flower stalk shows at its upper end a ring of five vascular cords. There passes out from the mid-point of each of these cords a strand which becomes a sepal midrib, and from each flank another branch which unites at once with a corresponding branch from the flank of the neighbouring cord to form a compound strand which becomes the vein marking the junction of two adjoining sepals. This vein is thus shown to correspond to two fused marginal veins. (This is also the case in *Oxalis*, see Fig. 5.) The emergence of the next whorl of five bundles for the petals breaks up the residual ring into ten masses from which there arise again two successive whorls of five bundles for the antesepalous and antepetalous stamens. The residual vascular tissue destined for the gynoeceum now shows a symmetrical arrangement of fifteen bundles, two in line with each other on the one set of radii, which become the midrib and fertile placental strand respectively of the antesepalous carpels, and a single undivided bundle (antepetalous carpel midrib) on the alternate

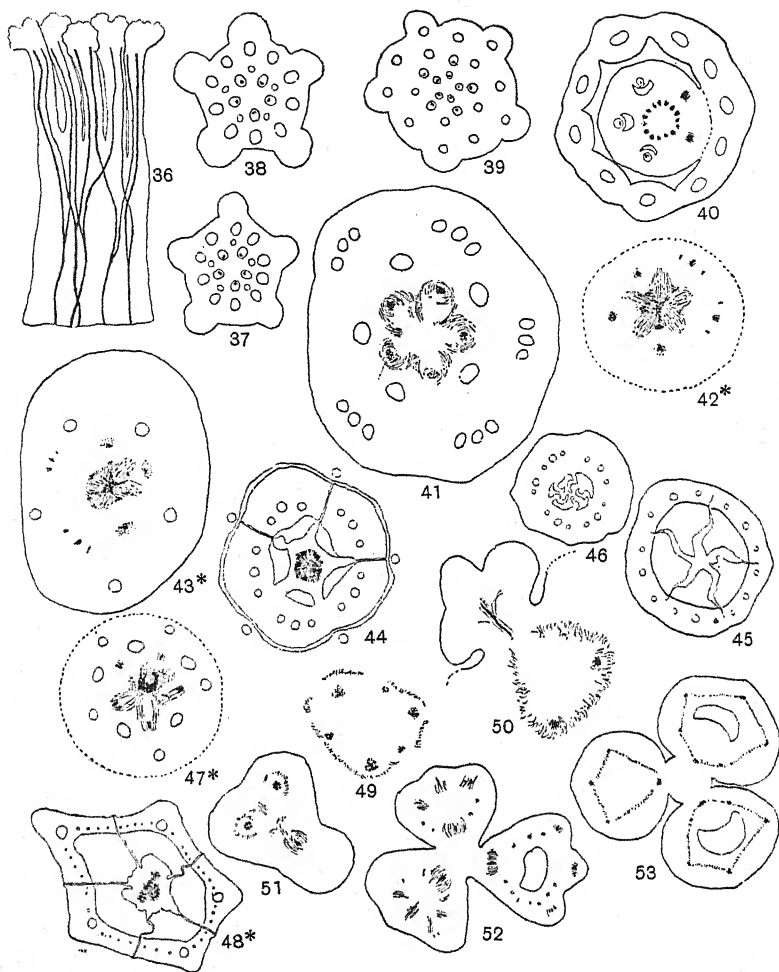
radii. But little xylem is left for these latter bundles, most of it having been appropriated by the antepetalous stamens, nevertheless they pass out to the periphery and develop a pair of lateral veins in the ovary wall. These species of *Biophytum* thus represent a grade corresponding in *Oxalis* to that of *O. corniculata* (*atropurpurea*), and they afford material in which the sequence of events in the vascular cylinder can be followed with the utmost ease.

#### BALSAMINACEAE

*Impatiens*  $G5 + 5 + 5$  (hitherto regarded as  $G5$ ) (Figs. 41-48).

In the Balsaminaceae we meet with yet another variant of the 4-whorled pentamerous ground-plan comprising androecium and gynoecium. There is here no trace of the second whorl of stamens. After the successive formation of sepals, petals and antesepalous stamens there arise in proper alternation the members of the gynoecium, which shows five antepetalous loculi and stigmas. These appearances have been looked upon as indicating once again in this alliance the presence of a single carpel whorl, but as will be seen, this interpretation cannot be reconciled with the anatomical evidence, which makes it clear that the inner stamen whorl has here been replaced by a carpel whorl. That is to say, instead of the usual  $A5 + G5, 5 + 5$  we find  $A5, G5 + 5 + 5$ .

After the emergence of the vascular cords for the five antesepalous stamens part of the residual vascular tissue becomes concentrated on the alternate radii, where it forms five well-marked xylem-containing cords (Figs. 41, 47). These cords become the midribs of the outermost whorl of carpels, and each gives rise at once, below the loculus level, to a pair of lateral veins (Figs. 42, 43). These carpels assume the valve form and are sterile. The remainder of the vascular tissue is now seen as five phloem rays situated on the antesepalous radii and meeting in the centre, where they form a ring encircling a confused mass of xylem which entirely obliterates the pith (Figs. 42, 43, 47). A rearrangement within this solid central core leaves only phloem on the antesepalous radii, while such of the xylem as is not discarded is concentrated into an inner ring of five strands on the radii of the petals (Fig. 44). As the outermost valve carpels expand, and the loculi make their appearance, the boundaries of these outer members become strictly defined not only on their inner face but also along their radial surfaces (Fig. 44). On the alternate (antesepalous) radii five septa come into being, reaching at their outer limits to the surface between the valve carpels and



Figures marked with an asterisk (42, 43, 47, 48) are reproduced on an enlarged scale in Plate IV facing p. 212.

Figs. 36-53. All from transverse sections except 36. 36. *Oxalis carnosa* Molina. Style column from a mid-styled flower pressed flat after being rendered transparent showing the division into two of each antesepalous carpel midrib and the further course of the two branches to the style filaments to right and left respectively in each case. 37-39. *Biophytum dormiens* G. Don. 37. The flower base above the level of exsertion of the sepals and petals showing the circle of ten cords for the androecium and the residual vascular ring serving the gynoecium consisting of five antesepalous cords with xylem and five antepetalous cords without xylem. In line with the antepetalous stamen cords and forming projections in the outline are the glands accompanying these stamens. 38. The same at a higher level. 39. The same after the antesepalous carpel cords have branched to form the midrib and placental bundles. 40. *Averrhoa bilimbi* L. The flower above the level of exsertion of sepals and petals showing the ring of ten conjoined stamens surrounding the ovary with which it is still fused on one side (right). In the ovary three antepetalous carpel cords have

extending to the central vascular core, which on these radii consists entirely of phloem (Figs. 44, 48). These dissepiments with the residual phloem standing in line with them are comparable with the septa (consolidated carpels) in the other related families, e.g. Linaceae, Oxalidaceae, and, we must suppose, here represent a second carpel whorl in which all xylem has been lost. But whereas in the Oxalidaceae two carpel whorls complete the carpel complement, we find left over in *Impatiens* a further set of xylem-containing cords on the radii of the petals and valves. These cords furnish the strands to the funicles, and constitute a third carpel whorl, the members of

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moved out towards the periphery and turned upwards, and are seen cut transversely at the outer border of the corresponding loculi; the two others are seen cut longitudinally as they make their way to the periphery. The antepetalous carpel cords are not yet differentiated. 41-46. *Impatiens tricornis* Lindl. 41. The flower base (sepal cords not shown). The five cords, already branched, destined for the petals and the five cords for the stamens are seen outside the central vascular ring in which the five xylem masses for the antepetalous, valve carpel cords are already differentiated. 42. The area of tissue which goes to form the gynoecium after these five carpel cords have turned outwards. Two of these cords have already formed a pair of lateral veins and are seen cut longitudinally (on the right). The central ring now shows five masses of undifferentiated vascular elements on the alternate (sepal) radii and five xylem groups on the valve (petal) radii. In the centre discarded xylem elements displacing the pith. 43. The conjoined androecium and gynoecium of another flower showing a slightly older stage. 44. The gynoecium now disjoined from the inner face of the androecium ring which is shown together with the five stamen cords. On the petal radii the outer sterile valve carpels with midrib, lateral veins and loculi, and nearer the centre, the inner, solid, fertile carpel cords. On the sepal radii, the second (middle) carpel whorl consisting of the narrow strip of ground tissue and epidermis between the valve carpels extending inwards as the septa together with the undifferentiated vascular tissue on these radii. 45, 46. The same at successively higher levels. The middle carpel whorl no longer extends to the surface between the valve carpels but is completely enclosed by the outer whorl. The vascular tissue of the innermost fertile carpel whorl is not shown [41, 43, 44 from one flower, 42, 45, 46 from another specimen]. 47, 48. *I. Roylei* Walp. 47. The area of tissue containing the vascular cords for the five petals, five stamens and the gynoecium. Those for the five outer carpels contain xylem and are seen cut longitudinally as they turn out from the central cylinder [a slightly earlier stage than that represented in 42]. 48. The gynoecium showing the five outer valve carpels, the second whorl forming the septa and still extending to the surface of the ovary, and in the centre the cords for the third whorl, two of which (below on the right) are not yet distinct [a stage between those represented in 44 and 45]. 49, 50. *Tropaeolum majus* L. 49. The residual vascular ring from which the carpel cords are differentiated showing the three large xylem bundles for the fertile valve carpels and three smaller bundles of xylem for the inner degenerating carpel members. 50. Older stage of the same. One of the outer carpels has begun to take on the valve form. The xylem of the inner whorl is disappearing. 51, 52. *T. peregrinum* L. 51. The ovary base showing the three valve carpel cords and some discarded undifferentiated vascular elements in the pith. There is no differentiation of definite cords on the alternate radii. 52. The ovary at the level of formation of the loculi. The outline as well as the vascular arrangement indicate that the inner carpel whorl has been entirely suppressed. 53. *T. pentaphyllum* Lam. The same region as that represented in 52. The valve carpels show midrib and strong secondary veins in addition to the fertile marginal veins. The small alternate bulges in the outline may represent the last traces of the lost inner whorl.



which are consolidated and fertile. When the fruit is ripe the outer ends of the dissepiments (second carpel whorl), which in the upper region of the ovary no longer extend between the first whorl as far as the outer surface (Fig. 45), tear at the point of junction with the valve carpels, so that the latter become detached, from below up, from the central column of the conjoined inner whorls. At the apex the innermost whorl comes to an end, leaving the members of the middle whorl with their inner borders free (Fig. 46). These in turn become gradually shorter in the radial direction (Fig. 46) until they, too, cease and the valve carpels alone continue upwards to form the short style column.

*Tropaeolum* G3 with or without remnants of the vascular tissue of a second whorl of three carpels (Figs. 49-53).

As stated in the earlier account<sup>1</sup>, the gynoeceum of *Tropaeolum majus* L. shows at the base six vascular cords (Fig. 49) (also noted earlier by Eichler)<sup>2</sup>, three of which supply the outer valve members and three, the alternate whorl now almost lost<sup>3</sup>. Here it is the valve carpels which are fertile; the inner members are sterile and are disappearing, for at a higher level in *T. majus* (Fig. 50) it is not unusual to find that the xylem has been lost from one or more of these members, while in *T. peregrinum* L. (canary creeper) the whole of the residual xylem is used up in the formation of the three valve carpels (Fig. 51). The surplus phloem ramifies in the pith and is discarded, so that before the level of the loculus floor is reached all trace of the inner carpel whorl is lost (Fig. 52). It is possible that in the contour line of the cross-section of the ovary of *T. pentaphyllum* Lam. we again find evidence of the existence of the three disappearing carpels, for the three valve carpels alternate with three smaller bulges which form the column from which the valves when ripe become detached. But if so, although retaining their outline, these sterile members have lost all trace of vascular tissue.

One unusual feature in all three species is deserving of notice, viz. the character of the veining of the valve carpels. These show one or more pairs of strong lateral veins between the midrib and the margins in addition to the ordinary well-marked, fused, fertile, marginal veins. We see in fact in the carpels here an approach to the palmate type of venation so conspicuous in the foliaceous leaves.

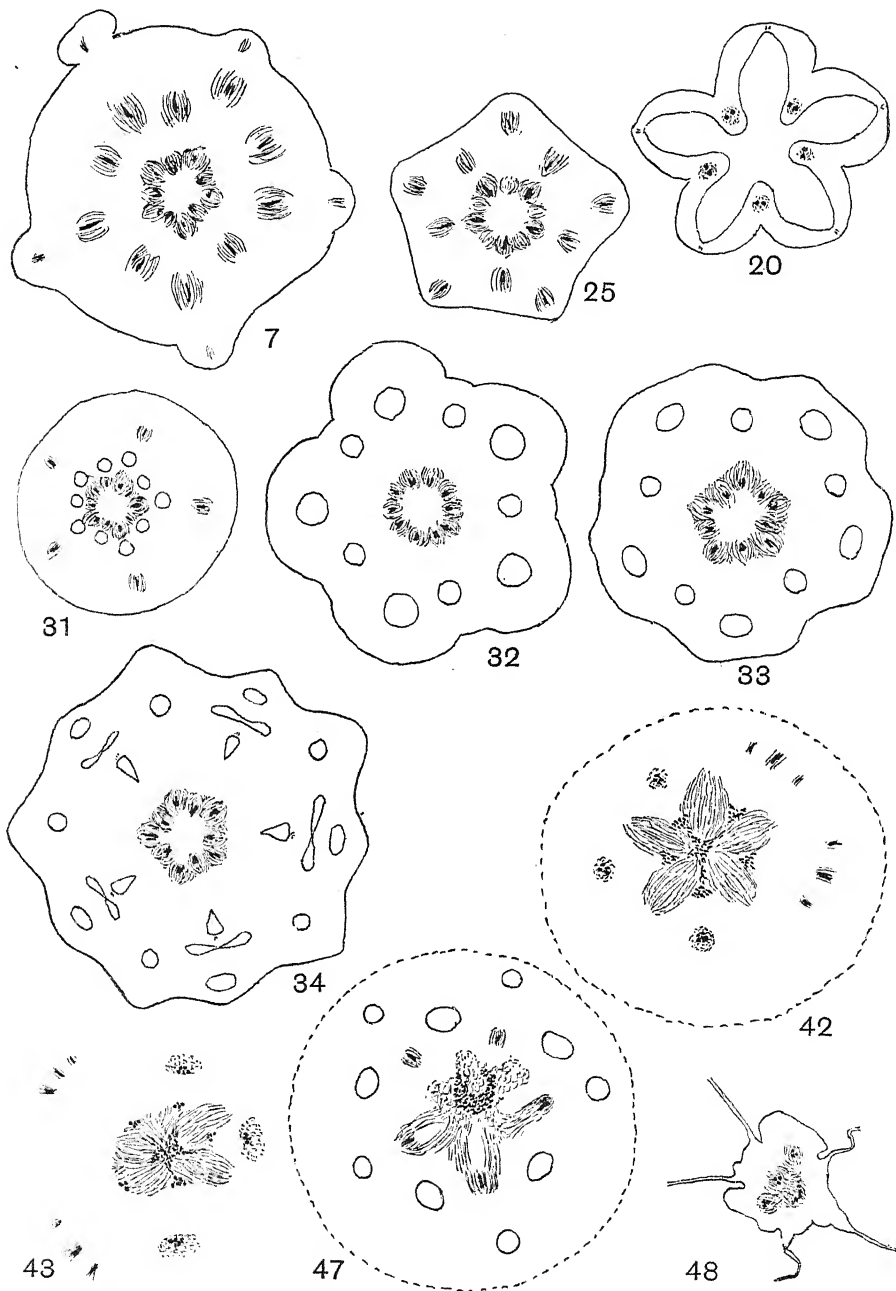
From the foregoing account it will be clear that it is now possible to view the positional relations of the members of the androeceum and

<sup>1</sup> *Ann. Bot.* 39, p. 149, 1925.

<sup>2</sup> *Blüthendiagramme*, II, Fig. 122, p. 197.

<sup>3</sup> Commissures of Eichler.





Enlargements of certain figures from pp. 204 and 212 to show clearly the details of the vascular strands



gynoecium in Geraniales as an intelligible and connected story. Stamen position is the outcome of carpel behaviour. Diplostemony, the (apparently) single whorl of ten members, or obdiplostemony results according as the cords of the five antesepalous carpel members alone, those of all ten members, or those of the five antepetalous members alone, leave the centre and curve outwards to permit the formation of the loculi. The presence in the style filament of a single median vascular bundle or of a pair of lateral strands with or without a third median strand is significant of whether a single whole carpel, or a combination of  $\frac{1}{2}$  &  $\frac{1}{2}$  carpels has been utilised in its formation.

#### SUMMARY

We may then sum up the position in regard to the Geraniales as follows. The full ground plan of the flower consists of six whorls which are usually pentamerous. This ground plan is realised in most Geraniaceae, Limnanthaceae, Linaceae, Oxalidaceae, Balsaminaceae. In all but the Balsaminaceae the androecium is composed of two whorls and the formula is then  $K_5, C_5, A_5 + 5, G_5 + 5$ . In the Balsaminaceae where the androecium is 1-whorled the fourth floral whorl develops into carpels, so that here the flower has the formula  $K_5, C_5, A_5, G_5 + 5 + 5$ . Six whorls are also present in the Tropaeolaceae, but here there is reduction of the number of members in both whorls of the androecium ( $4 + 4$ ) and of the gynoecium ( $3 \pm 3$ ). This scheme with the corollary that the carpels are polymorphic enables us to understand and to correlate the differences in floral structure in these several families, differences which, on the old monomorphic view, appear to be merely so many fortuitous dispositions, not governed by any discernible general principle. These diverse relations are exhibited in tabular form overleaf, together with the interpretation which follows naturally from the polymorphic standpoint.

The drawings accompanying this paper were made by Miss D. F. M. Pertz, to whom I desire to express my grateful thanks.

I am also much indebted to the Director of the Botanic Garden, Singapore, for material of *Averrhoa*.

Genera	Characteristics	Interpretation
<i>Geranium</i> <i>Erodium</i> <i>Pelargonium</i> (Geraniaceae)	Loculi 5, antepetalous Stamens obdiplostemonous Stigma arms antepetalous, each with one median vascular cord	G 5, antesepalous, sterile, solid, remaining central +5, antepetalous, fertile, valve, hence the obdiplostemonous condition Both whorls of carpels prolonged upwards to form the "beak," but the valve carpel cords alone continued beyond into the style column and the stigma ray, hence the antepetalous position of the latter, and the single vascular strand G 5, antesepalous, fertile, valve +5, antepetalous, sterile, solid, remaining central, hence the diplo- stemonous condition The antepetalous, sterile carpel cords alone prolonged into the style column and stigmas, hence the antepetalous position of the latter and the single vascular strand in each style component G 5, antesepalous, fertile, solid (or, expanded to become semi-solid), remaining central +5, antepetalous, sterile, valve (or, contracted to become solid), leaving the centre, hence the obdiplostemonous condition. (For the exceptional case of <i>Oxalis</i> <i>Ornigifolia</i> see p. 207) The antesepalous carpel cords prolonged into the style filaments after dividing in each case, so that the two halves enter the antepetalous style filament to right and left, hence the two strands in each filament and the bifurcate form of the stigma The antepetalous carpel cords also prolonged into the filaments in <i>Averrhoa</i> , but in <i>Oxalis</i> and <i>Biophytum</i> they are either untraceable or are reduced to a fine undifferentiated strand. Each style filament therefore represents $\frac{3}{8} \times 1 \frac{1}{8}$ carpels
<i>Linum</i> (Linaceae)	Loculi 10 (at the base) Stamens apparently forming one whorl of 10 Style and stigmas antepetalous	G 5, antesepalous, fertile, consolidated, leaving the centre +5, antepetalous, sterile, consolidated, also leaving the centre, hence the appearance of one whorl of 10 members in the gynoecium, with, consequently, a similar appearance in the androecium The antepetalous carpel cords prolonged undivided into the styles, hence their antepetalous position and the single vascular strand in each G 5, antepetalous, sterile, valve +5, antesepalous, sterile, consolidated +5, antepetalous, fertile, consolidated G 3, valve, fertile + traces of 3, solid, sterile ( <i>T. majus</i> L., ? <i>T. penta-</i> <i>phyllum</i> Lam.) or G 3, valve, fertile, only ( <i>T. peregrinum</i> L.)
<i>Limnanthes</i> (Limnathaceae)	Loculi 5, antesepalous Stamens diplostemonous Stigma arms antepetalous, each with one median vascular cord	
<i>Averrhoa</i> <i>Oxalis</i> <i>Biophytum</i> (Oxalidaceae)	Loculi 5, antepetalous Stamens obdiplostemonous Style filaments antepetalous, either with three distinct vascular strands ( <i>Averrhoa</i> ) or typically with only two lateral strands ( <i>Biophytum</i> , <i>Oxalis</i> )	
<i>Impatiens</i> (Balsaminaceae)	Loculi 5, antepetalous Stamens 5, antesepalous Stigmas 5, antepetalous Loculi 3 Stamens 4 +4 Stigmas 3, standing over the valves	
<i>Tropaeolum</i> (Tropaeolaceae)		

# OBSERVATIONS ON *RHYNCHOSPORIUM SECALIS* (OUD.) DAVIS, LEAF BLOTCH OF BARLEY AND RYE

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(With Plate V, and 3 figures in the text.)

THE occurrence of *Rhynchosporium Secalis* in Britain was first recorded in 1919 by Cotton(1), who stated that it had been found on rye in Devon and Cornwall. Since then the present writer has had this fungus constantly under observation in the neighbourhood of Cambridge, where it occurs frequently on barley and to a lesser extent on rye. As it was considered likely at one time that the fungus might prove destructive to certain new hybrid barleys produced on the University Farm, an investigation of the disease caused by it was undertaken.

The fungus was first described in 1897 by Oudemans(2) in Holland under the name *Marsonia Secalis* Oud., and in the same year Frank(3) referred briefly to a disease of rye and barley in Germany caused by the same fungus. The name was changed later to *Marssonina Secalis* (Oud.) Magnus. In 1900 Heinsen(4) in Germany transferred the fungus to the new genus *Rhynchosporium* because of the peculiar beaked, once-septate spores, and named it *Rhynchosporium graminicola* Heinsen. Lastly, Davis(5) in the United States named it *Rhynchosporium Secalis* (Oud.) Davis, in accordance with the International Rules of Nomenclature, and it is by this name that the fungus is now generally known.

In addition to its presence in Holland, Germany, the United States and Britain *R. Secalis* has been recorded in Canada by Drayton(6), and in New South Wales(7), where several types of fodder barleys have proved resistant to it. The disease, although most prevalent on barley and rye, has been seen sparingly by Heinsen(4) on wheat in Germany, and on a considerable number of wild grasses, including *Dactylis glomerata*, *Agropyrum repens* and *Bromus inermis*, in various countries. Apart from an attack on rye seen by Heinsen(4) and a statement by Drayton(6) that the disease is serious in the

Mississippi Valley, no considerable damage to crops has been attributed to this fungus. In California it is stated(8) that *R. Secalis* attacks barley, especially early sown varieties, soon after germination in the autumn, but that some varieties are resistant to attack.

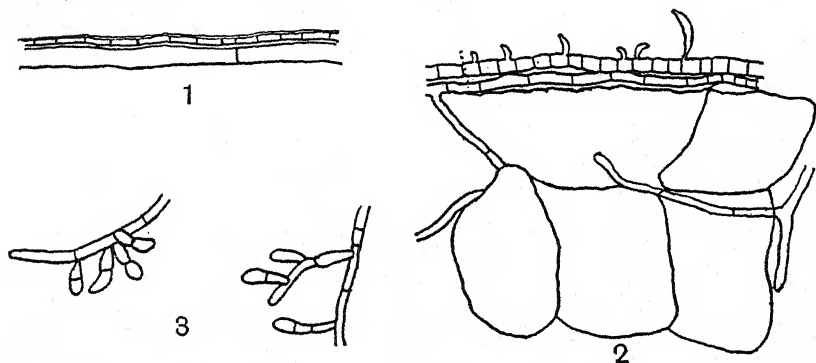
In the neighbourhood of Cambridge *R. Secalis* has been found by the writer and by Professor N. J. G. Smith on *Bromus sterilis*, *B. mollis* and *Dactylis glomerata*, as well as upon barley and rye.

Around Cambridge the disease is most common on barley during the latter part of the winter and early spring, especially when winter-sown. It is also abundant on self-sown barley plants during the autumn. During early spring some new hybrid barleys on the University Farm have been severely affected, but when active growth commences at a later stage the plants grow away from the disease, and the foliage subsequently formed is almost free from attack.

*R. Secalis* affects any part of the leaves, producing spots or blotches of irregular shape (Pl. V, figs. 1 and 2). On barley the auricles are commonly attacked, perhaps because of the tendency for water to be held in this region. The blotches first appear as water-soaked areas, which become greyish in the centre with a brown margin. The blotches often coalesce and entire leaves may be destroyed by the fungus. The greyish colour in the middle of a blotch is due to the formation of spores, which are produced over nearly the whole area and lie on the surface at maturity. Spores may be formed on both surfaces of the leaves.

The spores are typically two-celled and markedly beaked, as described by Heinsen(4). In my experience spores from barley leaves measure  $11-16 \times 3.5-5\mu$ . According to Heinsen(4), in the process of spore formation, hyphae become entangled together under the cuticle and give rise to short branches which penetrate it and form spores, but he gives no figure of the process of spore formation on the leaves. The illustrations of spore formation given by him concern this process in cultures. Hand sections through the blotches showed that Heinsen's description of the mode of spore formation on the leaves was incomplete, so material was fixed and microtomed in order to make out the exact method of spore formation. As a preliminary to spore formation certain hyphae penetrate the epidermis from the mesophyll and arrange themselves, partly under the cuticle (Text-fig. 1) and partly in the epidermal cells, so that they lie parallel with the surface of the leaf. These hyphae become divided up by transverse septa into small, regular cells (Text-figs. 1 and 2). These cells then put out small protuberances

by a process of budding, which rupture the cuticle (Text-fig. 2). The protuberances become curved and once-septate, and at maturity are separated off from the parent cells as spores. This mode of spore formation is very peculiar and the writer knows of no other parasitic fungus in which it occurs. From this description it can be readily understood why the whole of the central region of the blotch appears to be covered with spores. Davis(5) states that the mode of spore formation in *R. Secalis* is the same as in *R. Alismatis* (Oud.) Davis, where undifferentiated hyphae make their way to the stomata and bear conidia at their extremities. The present writer has not seen conidiophores of this fungus in the stomata and there is no doubt that the great majority of the spores, if not all of them, are formed in the peculiar manner described above.



Text-fig. 1. Mycelium of *Rhynchosporium Secalis* under cuticle of barley leaf.  $\times 350$ .

Text-fig. 2. Mycelium of *Rhynchosporium Secalis* in barley leaf showing spore formation (the cuticle has broken away).  $\times 450$ .

Text-fig. 3. Spore formation of *Rhynchosporium Secalis* in a hanging-drop culture on Dox's agar.  $\times 350$ .

The spores germinate readily in water, each cell putting forth one or more germ tubes. In hanging-drop cultures on Dox's medium or barley-extract agar the germ tubes form a sparse mycelium which quickly gives rise to spores from unspecialised cells, as indicated in Text-fig. 3. In cultures the spores are curved or straight, mostly uniseptate, but occasionally 2 to 3-septate. No indication of a yeast-like mode of development as described by Heinsen(4) was observed in the writer's cultures. The fungus does not grow well on agar media. In plate cultures the colonies are of strictly limited growth (Pl. V, fig. 3); at first they are greyish in colour, but become brownish with age; spores are produced on the surface of the colonies, the mode of

formation being the same as in hanging-drop cultures, and as described by Heinsen(4). On the other hand the fungus grows well on barley leaves sterilised by heat, and spores are formed freely on them.

Inoculation experiments carried out during the winter showed that infection of unwounded barley leaves could be brought about by placing an emulsion of the spores in water on either surface of the leaf. Heinsen(4) also successfully reproduced the disease by inoculation.

No other spore stage than that described has been observed in the life-history of this fungus. Heinsen(4) states that the fungus possesses some capacity for survival in the soil, but no evidence for this has been observed by the writer. There is no difficulty in understanding how the fungus survives from season to season. The fungus is not much in evidence during the height of the summer, but it frequently becomes abundant on self-sown barley plants in the autumn. As previously indicated, *R. Secalis* occurs on a considerable number of wild grasses, and these undoubtedly may be a source of infection to crop plants.

It is very difficult to estimate the amount of damage caused by this fungus. During the latter part of the winter and very early spring certain new varieties of barley on the University Farm have been seriously affected by *R. Secalis*, the lower leaves of these plants being almost completely destroyed by it. So gravely affected were these new varieties that they were in danger of being discarded by the plant breeders who had produced them. With the renewal of active growth in April and May even the most severely affected types recovered to such an extent that at harvest time little difference could be discerned between them and varieties which had been only slightly attacked. The loss of foliage, however, must have had some weakening effect on the plants.

I am indebted to Professor N. J. G. Smith and Mr W. C. Moore for references.

#### SUMMARY

1. Leaf Blotch of barley and rye, caused by *Rhynchosporium Secalis*, is of common occurrence in the vicinity of Cambridge, being most frequently seen in late winter and early spring. The fungus occurs also on various wild grasses.
2. The disease is characterised by blotches of irregular shape on the leaves. At maturity these discoloured areas are greyish with a brown margin.



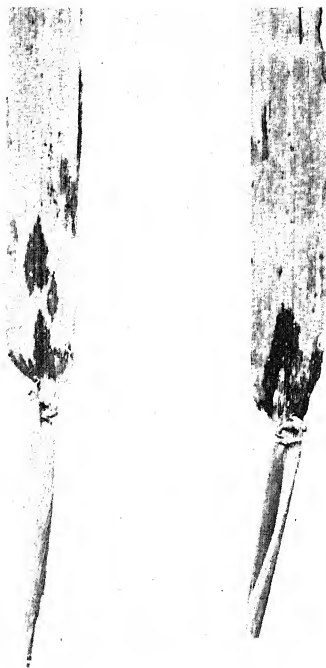


Fig. 1



Fig. 2

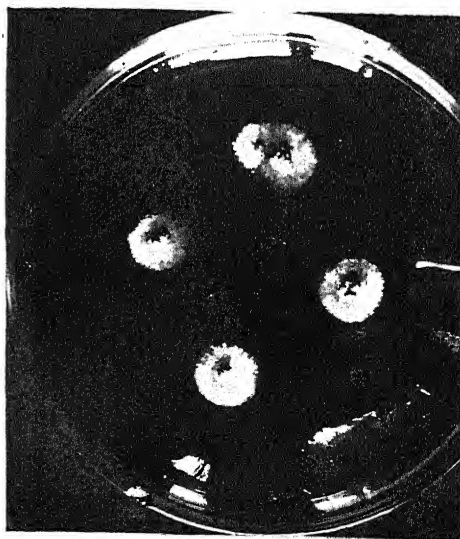
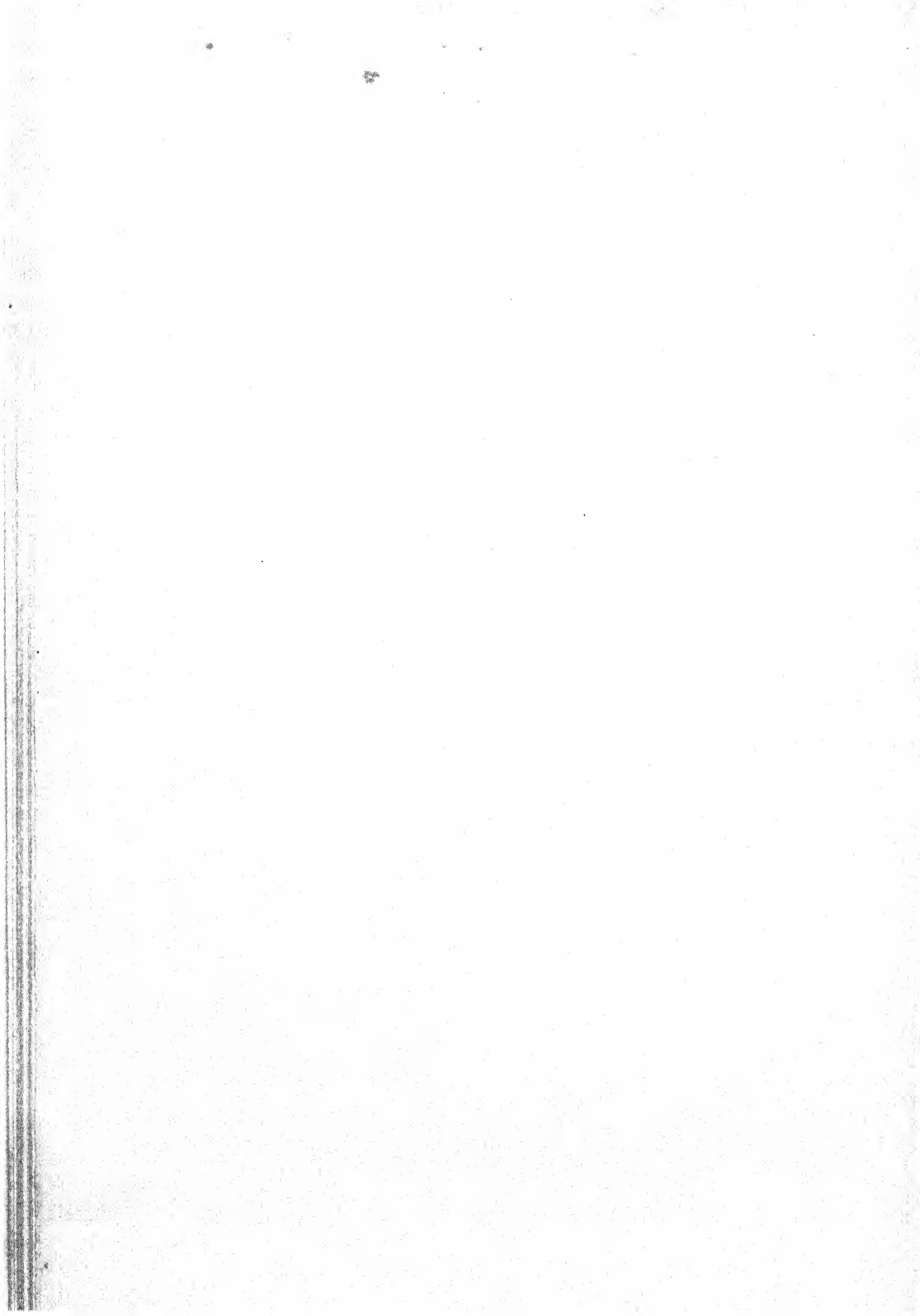


Fig. 3

Fig. 1. Blotches on barley leaves caused by *Rhynchosporium Secalis* (nat. size).

Fig. 2. Blotches on barley leaves caused by *Rhynchosporium Secalis*: older stage, at the time of spore formation ( $\frac{2}{3}$  nat. size).

Fig. 3. Colonies of *Rhynchosporium Secalis* on barley-extract agar ( $\frac{2}{3}$  nat. size).



3. It is unlikely that serious damage will be caused by this fungus under English conditions as even the most susceptible types of barley grow away from the disease when active growth commences in the spring, the later formed leaves being almost free from infection.

4. The peculiar method of spore formation of *R. Secalis* on the host is described. Profusely septate hyphae arrange themselves under the cuticle and in the epidermal cells, parallel with the surface of the leaf. The cells of these hyphae bud forth protuberances which disintegrate the cuticle and become exposed as spores. The method of spore formation differs considerably from the accounts of this process given by Heinsen (4) and Davis (5).

5. Inoculation experiments show that both surfaces of the leaf can be infected by this fungus without wounding.

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## A MODIFIED FORM OF AUTO-IRRIGATOR

By G. REDINGTON, M.Sc.

(With 7 figures in the text.)

THIS short paper describes a method by which the roots of potted plants can be assured of a regular and practically unvarying supply of water over a considerable period of their life. The apparatus used is simple, cheap, and easy to construct.

In the first place acknowledgements must be made to Professor J. H. Priestley, with whom the idea of this modified form of auto-irrigator originated.

It was decided to use the apparatus in connection with experiments on the growth of plants in continuous light, in order that the plants under investigation could be supplied with water by this means.

The general practice of watering plants grown in pots is to withhold water until the soil becomes dry, as indicated either by the appearance of the soil surface, the weight of the pot, or the hollow sound emitted when tapped. The pot is then filled with water and the process repeated. This method obviously has many disadvantages both for ordinary and special circumstances, and the supply of water to the roots in this way is far from being regular. The modification of this method sometimes adopted, where measured quantities of water are supplied at regular intervals, also has disadvantages and can have little relation to the actual needs of the plant.

In the growth of plants in continuous light it was important that all other external conditions should be kept as uniform as possible in order that no factor should operate tending to introduce any periodicity into the life of the plant. The auto-irrigator, it is claimed, is a method by which the supply of water to the soil is controlled by the rate at which it is being lost by evaporation from the surface and sides of the pot and by transpiration. Thus the supply to the plant is regulated primarily by its needs, and the soil moisture content remains practically constant. If this end is to be attained it follows that the water-supplying power of the apparatus must be greater than any demand which is to be made upon it.

The idea of the auto-irrigator was apparently first developed in America, and an early apparatus was described by Livingston(4) in 1908 whose short paper was followed by one from Hawkins(2) with

a further paper from Livingston(5) in 1918. In this case the water supply to the roots of the plant was provided by one or more porous porcelain cups buried in the soil of the plant pot. Originally these cups were cylindrical in shape and similar to the atmometer cup, but later conical cups were used in order to maintain better contact between the soil and the surface of the cup. The cup is filled with water and connected by a rubber stopper and a tube to a water reservoir at a lower level. As water is taken from the soil by plant roots or by evaporation, more water moves from the wall of the cup into the soil, the cup being replenished by water passing up to it from the reservoir. Holmes(3) showed that moisture equilibrium in the soil of pots equipped with auto-irrigators of this type was attained in 75 to 90 days, according to whether a clayey or a sandy soil was used. Livingston and Hawkins(6) found that in this way a fairly constant soil water content is maintained over a period of 24 hours, the maximum plus and minus variation from the mean in six pots being 4.6 per cent. of the mean moisture content of the soil on the basis of weight of dry soil.

The modified form of apparatus to be described is like the porous cup auto-irrigator only in that the soil of the pot is in contact with a water supplying surface which is itself automatically kept supplied with water.

#### DESCRIPTION OF APPARATUS

Suitable sized pots are selected (a little larger than would normally be used) and the drainage hole enlarged to a circular hole, varying in size with the size of the pot. Usually a hole  $\frac{3}{4}$  in. in diameter is sufficient for a 3-inch pot<sup>1</sup>,  $1\frac{1}{4}$  in. for a  $4\frac{3}{4}$  to 5-inch pot and  $1\frac{1}{2}$  in. for a 6-inch pot. A piece of ordinary grey flannel is cut to correct size and sewn up to form a cylinder which will fit closely inside the pot. A thread is run through the flannel, drawing it up at the point where it will pass through the enlarged drainage hole, leaving the top of the flannel level with the rim of the plant pot. The pot is thus lined with the flannel, which is continued down through the drainage hole and out in the form of a 'wick' several inches long. The drainage hole is then stopped up by screwing a rubber stopper tightly into the flannel from below. The pot is then ready for the reception of the soil and plant or seeds. After planting, the pot is stood in the neck of a large glass jar which is kept filled with water to within an inch or two of the bottom of the pot. Flannel

<sup>1</sup> Sizes of pots expressed as internal diameter in inches.

was used because it is cheap and has considerable powers of water absorption when once wetted thoroughly. It is essential that the flannel and the pot be moist before being filled with soil. Stoppage

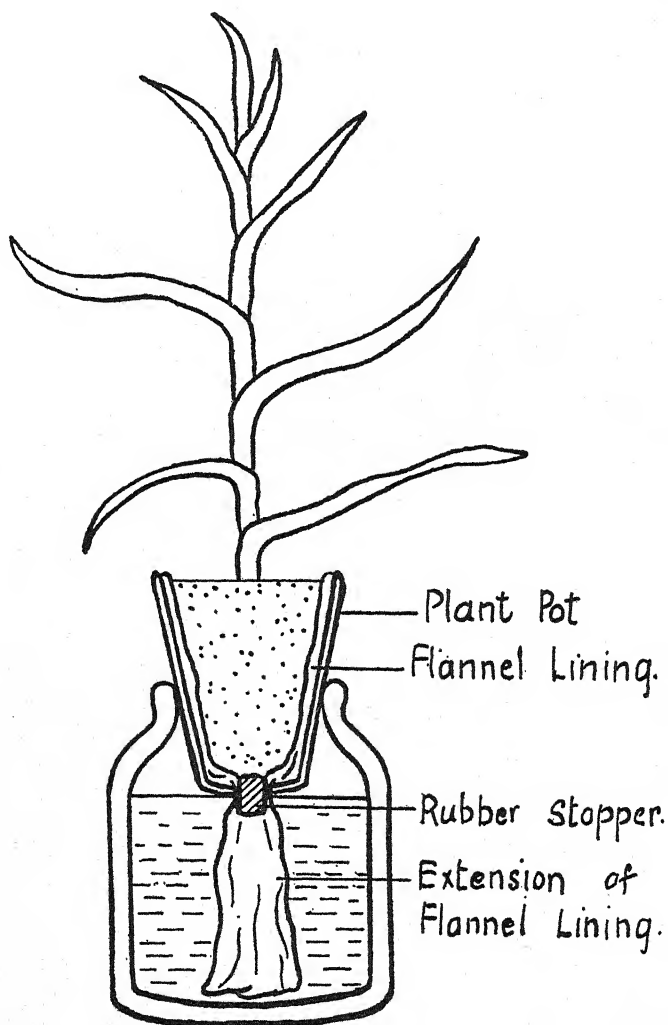


Fig. 1.

of the drainage hole with a rubber bung was found to be the only way of preventing the roots from growing out through the hole and into the water below, and it was found that this did not interfere with the supply of water to the pot.

The amount of water supplied to the soil is controlled mainly by the relation between the area of the water-supplying surface—the flannel lining to the pot—and the volume of soil in the pot. As this volume increases, the proportion of surface to volume decreases, so that the smaller the pot the greater the supply of water. There will thus be a practical limit to the size of the plant pot that can be used, though what this limit is has not yet been determined. It will vary with the water requirements of the plant, the kind of soil used and the degree of consolidation of the soil in the pot. In practice the method has been used successfully for pots up to  $6\frac{1}{2}$  in. in diameter. Usually for large-sized pots containing plants which make a heavy demand on the water supply, it is advisable to use a double thickness of flannel, whilst a single layer has been found sufficient for pots up to 4 in. in diameter.

#### EXPERIMENTAL

The chief tests of the apparatus were made with a series of maize plants grown in pots very much smaller than would ordinarily be used for the purpose. As it was desired to put the capacity of the apparatus to the most severe test, it was decided to grow half of the test plants under continuous illumination, as under these conditions the practically unceasing loss of water in transpiration would throw a great strain on the power of the apparatus to supply adequately the abnormal needs of the plant. Space was available in a room 10 ft. by 5 ft., lighted by four Ediswan gas-filled electric lamps, each of 500 watts. The temperature of the room was approximately  $30^{\circ}$  C. and the average relative humidity 35. A vigorously growing plant such as maize under these conditions would demand a large supply of water. Six young comparable maize plants (var. Giant Caragua) were selected from about 50 seedlings, and potted, using ordinary potting soil that had been passed through a  $\frac{1}{4}$ -inch sieve. The pots were  $4\frac{1}{2}$  in. in diameter by 5 in. deep. The drainage hole was enlarged to a diameter of  $1\frac{1}{4}$  in. to accommodate the double thickness of flannel used for lining the pot, and stopped as usual with a rubber bung. The pots were then placed each in the neck of a glass jar which was filled with water to just below the bottom of the pot. Three of the plants were grown in continuous light (C 1, C 2 and C 3), and three in intermittent light (L 1, L 2 and L 3). Four of the plants were grown under experimental conditions for 15 weeks (from February 15th to June 12th). At the end of that time they had reached a height of from 6 to 8 ft., had borne and matured

terminal staminate inflorescences and were bearing the young pistillate inflorescences.

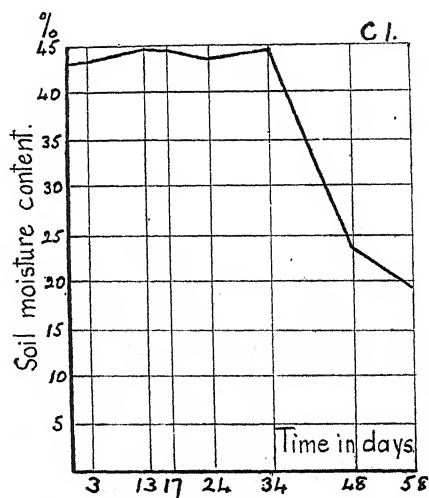


Fig. 2. Plant grown in continuous light.

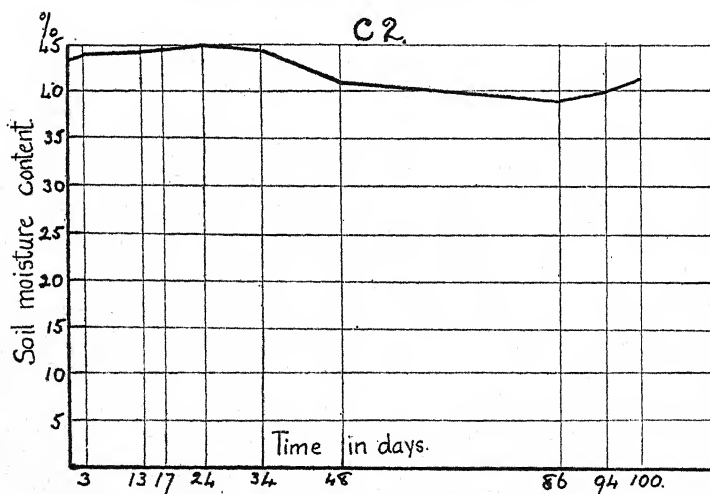


Fig. 3. Plant grown in continuous light.

#### DETERMINATIONS OF SOIL WATER CONTENT

Many determinations of the water content of the soil in the pots were made during this period, the first samples being taken 8 days after potting up the seedlings. The sampling tool used was a cork borer of 1.8 cm. diameter. All determinations were made in dupli-



cate, two samples being taken in each case from different parts of the pot. The holes were then filled up with soil taken from the bulk

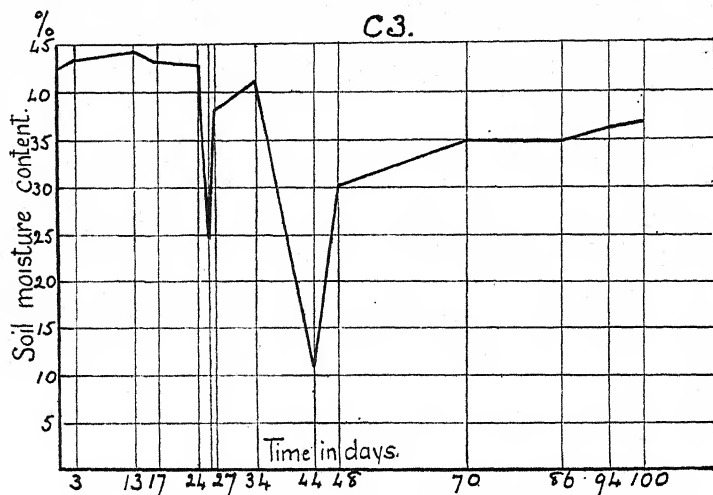


Fig. 4. Plant grown in continuous light.

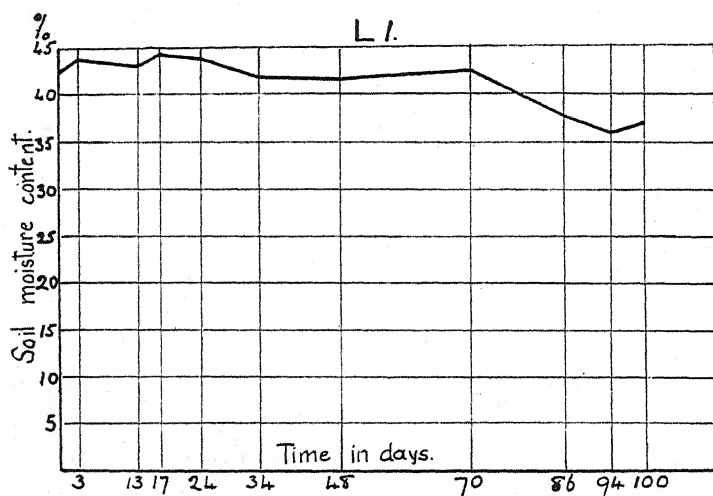


Fig. 5. Plant grown in intermittent light.

reserved from potting, every care being taken to ensure as nearly as possible the same degree of consolidation. The method used for determining the moisture content was that adopted by the Agricultural Education Association (1).

Possible variations in soil water content would be between  
(a) samples from different pots;

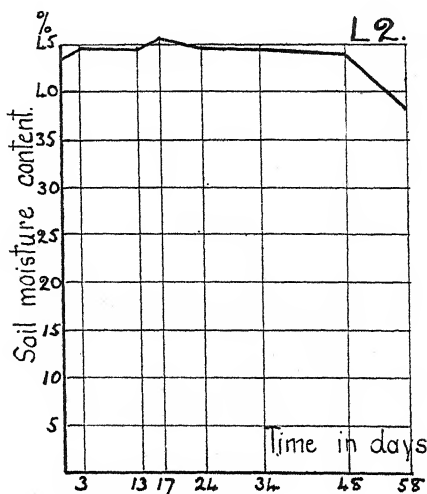


Fig. 6. Plant grown in intermittent light.

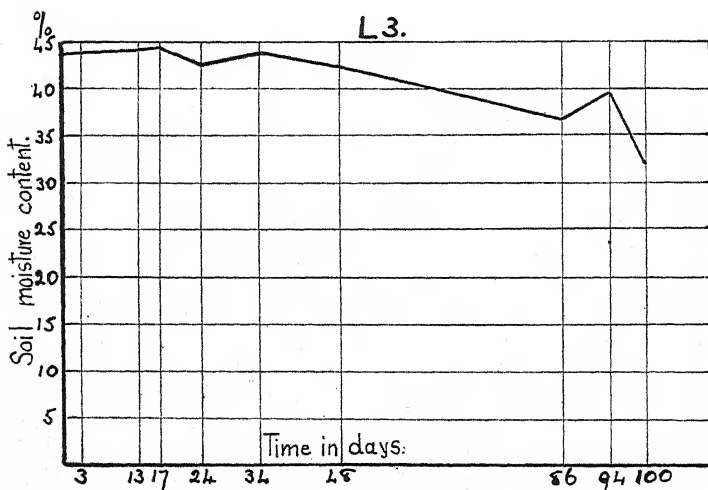


Fig. 7. Plant grown in intermittent light.

- (b) samples from different parts of the same pot;
- (c) samples taken at different periods during the growth of the plants;

- (d) samples taken with the water in the reservoirs at lower levels;  
(e) samples taken after the intermittent light plants had been in the light for 16 hours, and others taken after they had been in darkness for 8 hours.

Possible variations from these causes were investigated, with the following results.

(a) and (c). In the accompanying figures the soil water content for the six pots is shown graphically<sup>1</sup> over the whole of the experimental period of 15 weeks in four cases and 9 weeks in the other two. These latter plants were removed at the end of this time as they had grown to the roof of the experimental room, a height of 8 ft.

It will be seen that in four cases there was a close agreement between the different pots over the greater part of the growth period and it was not until the plants had reached full height and were beginning to flower that the soil water content began to fall. Plant C 1 made the most rapid growth and so was the first in which the demand for water exceeded the supply. It is only to be expected that such vigorous plants growing in small pots will in time reach the stage of requiring more water than the apparatus could supply. The breaks in the curve of plant C 3 are due to the fact that on these two occasions the pots were purposely left without water.

The dozens of samples from which these curves were plotted were taken on different dates at different times of the day between 8 a.m. and 9 p.m., the six pots generally being sampled together.

(b). The general distribution of water in the soil of the pots was investigated by comparing samples taken from different depths, and also samples taken at varying distances from the centre of the pot. These showed that there was a slight increase in water content from the surface to a depth of 9 cm., and also a slightly higher water content near to the flannel lining.

		Average water content of 6 pots (%)
Samples	0-3 cm. depth	42.7
"	3-6 "	44.8
"	6-9 "	45.8
"	close to flannel lining	44.8
"	near to centre of pot	42.4

These small differences cannot be of significance in considering the general supply of water to the plant roots.

<sup>1</sup> Soil water content throughout is calculated on wet soil.

(d). On two occasions the water in three of the containers was allowed to fall from 6-9 cm. below its usual level. The average water content for the pots supplied from the normal level was 44.6 per cent., and from the low level, 43.9 per cent.

(e). With the three plants in intermittent light, samples taken after the 8 hours' darkness showed practically the same water content as those taken after the 16 hours' light period.

After several weeks' growth the soil water content of one pot was twice allowed to drop, once to 24.6 per cent. and again to 10.7 per cent. On filling up the containers again, the normal water content was attained within three or four days.

#### GENERAL

This apparatus has been used with success for the growth of many species of plants, including such diverse types as *Zea mais*, *Gossypium herbaceum*, *Boehmeria nivea*, *Kleinia articulata*, *Maranta arundinacea*, *Canna indica*, *Vicia faba* and *Lapageria* spp., in pots varying in diameter from  $2\frac{1}{2}$  to 6 in. The chief precautions that have been found necessary in the use of this method of auto-irrigation are due to the fact that in the early stages of the life of a slowly-growing plant, there is a tendency for water to be supplied to the soil more freely than the plant requires, and in young, newly potted plants of this type this had resulted in the loss of 5 plants out of 45 grown. In view of this tendency it is advisable to observe the following precautions in the case of young, newly potted plants of slow growth.

1. A single thickness of flannel should be sufficient for all sizes of pots up to 5 in. in diameter.

2. The soil used for potting should contain a fairly high proportion of sand.

3. The soil should not be rammed too firmly about the roots, but should be left rather looser than is usual.

4. After potting up such young plants, allow two or three days for the soil moisture content to reach equilibrium, and then empty the containers and just keep the flannel moist until the plants have rooted freely in the fresh soil. When active new growth of leaves indicates that this stage has been reached, the containers can be kept filled with water in the usual way.

It will of course be remembered that there exists no free drainage in the pots, so all water entering has to be removed by evaporation and transpiration. Hence it is inadvisable and quite unnecessary

to "water in" newly potted plants or newly sown seed, as is usually done.

The apparatus will remain in working order as long as will be usually required for the growth of plants under either ordinary or experimental conditions, and one is now in use which has supplied a 6½-inch pot for over a year.

The cost of the electric light used in the experiments on the growth of these plants was met by a Research Grant from the Carnegie Trust for the Universities of Scotland.

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# THE INTER-RELATIONSHIPS OF THE ARCHIMYCETES

By W. R. IVIMEY COOK, B.Sc., PH.D., F.L.S.

(With Plates VI—VIII, III text-figures and 9 diagrams  
in the text.)

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## I. INTRODUCTION

IN any attempt to formulate a phylogenetic series of types which might have given rise to the various groups of organisms which form the lower fungi, it is necessary to consider a number of species which do not commonly find their way into botanical literature.

A vast number of organisms exist at the present time whose life histories have not been studied and very little attempt has been made to classify them. On the whole, botanists have left them alone on the ground that being devoid of chlorophyll they cannot be true plants. On the other hand it has never been proved that their mode of nutrition is holozoic, and the possibility that many of them are saprophytic or semiparasitic justifies their consideration as a basis for deriving some of the lower groups of fungi.

In these organisms motion is obtained either by the gradual flowing of the protoplast as a whole, or by the specialisation of some parts of it in the form of flagella. In some the protoplast may be extended in the form of pseudopodia which may either be blunt and but little differentiated from the main, in others these pseudopodia are very fine and show less and less tendency to retraction. In a more advanced condition we find the pseudopodia as permanent structures—flagella—only very rarely retracted and capable of independent motion. The body is more or less homogeneous and the protoplast does not become surrounded by a cell wall until the pseudopodia become restricted to a particular part of the body.

Little or nothing is shown in the majority of cases about the cytology, although in most of the more specialised members we find a more or less typical mitosis. As far as our knowledge of the group as a whole goes, there is some evidence that nuclear division is effected by other means besides that generally understood as mitosis.

Such an organism must either be primitive or the product of a reduction series, and of the latter we have no evidence at all. It therefore seems justifiable to consider such an organism as a simple one, and we are next called upon to consider its mode of nutrition. If we assume for the moment that it is holozoic and ingests other organisms, it naturally follows that it cannot itself be truly primitive, since some previous forms of life must have existed upon which it fed. On the other hand if it is considered to be saprophytic it could have existed without assistance from other organisms. So far as we know, bacteria are the only forms of life which may be more simple than these organisms. Their claims to simplicity will be considered later; suffice it to say at this stage that their extreme minuteness requires that their whole metabolism must be effected within a very restricted area, and this in itself suggests that many of them are not by any means simple. If, therefore, we eliminate the bacteria, we have no organisms upon which so far as we know these primitive forms could live, and furthermore, no bacteria have been demonstrated with any certainty within their soma. It seems reasonable to conclude that at any rate initially their mode of nutrition was saprophytic, and that they existed as saprophytes upon chemical substances, and in the case of the Iron and Sulphur Bacteria, not organic. From these substances they obtained energy either by an anabolic or a katabolic action.

Few of these organisms contain chlorophyll or any other green pigment, though in the more advanced members we do find chlorophyll present. Several cases, moreover, are on record of one or two species of a genus possessing green pigment while the rest are devoid of it. It is generally assumed that these latter have been derived from the form possessing pigment. Such a view is associated with the idea that chlorophyll only originated once in the course of evolution. In chemistry it is known that a coloured dye can be obtained from a colourless substance by a slight alteration in the molecule. It seems quite reasonable to postulate that the same might be true of chlorophyll, in other words, that a prochlorophyllin is present in the cells of many of these primitive organisms, and that only here and there has the final stage been effected resulting in the

production of a green colour. Furthermore there is some evidence from spectrum analysis that the composition of the green colouring matter of green protists differs (37). We know now that chlorophyll acts solely as a photocatalyst and that similar reactions can be artificially produced by using Malachite Green. Chlorophyll is not more complicated than many other proteins, yet it would be difficult to prove that all these have only been evolved once in the course of evolution.

From this consideration we see that there is evidence that the primitive ancestor was one that never possessed chlorophyll, though in the course of its evolution it developed substances which though colourless were nevertheless products from which chlorophyll, or something very similar to it, could be synthesised.

It is well known that haemoglobin is very similar in chemical constitution to chlorophyll. Haemoglobin, or red blood, is found first among the Annelida and Mollusca, yet in these phyla certain genera exist some of whose species possess haemoglobin while others do not, and it has been suggested that this lack of colouring matter is associated with habitat. These animals without haemoglobin may be reasonably looked upon, not as forms which have lost the red colouring matter, but as forms which have not yet completed the synthesis, and that the final stage required to convert the colourless blood into haemoglobin has not been evolved.

Of course it is quite possible to argue that organisms devoid of chlorophyll or haemoglobin have lost these coloured substances which they once possessed, but since we have clear evidence that they are advantageous to plants and animals respectively, since in both the more highly evolved groups possess them, there is, at any rate, some justification for the view that in those groups in which pigment first appears, those forms in which it is absent are forms which have not yet been able to complete the synthesis.

Returning now to the consideration of chlorophyll, if the colourless prochlorophyllin is present in many colourless forms, it follows in all probability that chlorophyll, or a similar green pigment, was produced more than once in the course of evolution. If this is the case, it will explain why certain isolated members of genera devoid of green pigment possess chlorophyll. Moreover, we find in several cases that species in which chlorophyll is present are more successful and widely distributed than are those species in which it is absent. One may instance the genus *Euglena* as an example of this, for



*Euglena viridis* is by far the most commonly met with species in the genus.

Such primitive organisms as have been described above are found at the present time, and are usually grouped in the Proteomyxa. Unfortunately the Proteomyxa of Haeckel has been merged into other groups by different workers; we find them included in the Labyrinthulideae by Parker, and in the Sarcodina by Lankester. Döflein considers that the Proteomyxa are a group of the Rhizopoda, which he takes as the simplest of the Protozoa. It is considered, therefore, that it is within the Proteomyxa that the genesis of plants and animals must be sought.

At the same time we have abundant evidence that loss of chlorophyll has occurred, though usually as a result of parasitism. Such forms would naturally have originated secondarily, since it would be necessary for a considerable evolutionary development to have occurred before there were any organisms sufficiently advanced to support parasites.

Among the primitive fungi it is considered therefore that two tendencies have been working, the one resulting in a series of saprophytic forms which may later have become parasites, and the other the parasitism of types which previously enjoyed a holophytic existence. Both these groups have developed along their own lines, resulting in the formation of genera and families which had a totally different origin, but which in the course of time, by parallel evolution, have come to resemble one another. Their parasitic existence would naturally, moreover, increase their degree of similarity. Furthermore, other apparently simple forms may have been derived from more complicated types by reduction due to parasitism.

In general there seems far more evidence in favour of a polyphyletic rather than a monophyletic origin of the primitive fungi, and the problem which presents itself is to try and differentiate between what is primitive and what has been secondarily evolved.

Inasmuch as the method of nutrition in most of these simple organisms is unknown, we find some groups mutually claimed by both botanists and zoologists, and similarly others mutually ignored. It is therefore desirable at this point to consider what groups of organisms should be included in what is generally designated as the Lower Fungi, to which the term Archimycetes is now applied. It must be admitted at the start that it is impossible to lay down any hard and fast rules at what stage in evolution an organism ceases to be a member of the Proteomyxa and commences to be classified

as either a plant or an animal. At the same time, there are certain well-defined groups of organisms which are sufficiently distinct to be classified among the Lower Fungi. It is proposed to include in the Archimycetes those forms which possess a more or less definite asexual reproduction by means of zoospores, together with, in the more advanced families, some method of sexuality which may be either isogamous or heterogamous. In other words, the Archimycetes will be considered to include the Mycetozoa, Plasmodiophorales, Acrasiales, Chytridiales and Peronosporales. The Zygomycetes stand apart from the other groups in many respects and it is difficult to relate them to the simpler forms.

Such a classification does away with the old term Phycomycetes which was considered to embrace the Zygomycetes and Oomycetes. It will be shown that it is possible to trace back the Oomycetes to a primitive protist ancestor, which does not exhibit any true sexual reproduction of the type characteristic of the higher Oomycetes, and that this primitive form is itself closely similar to the organisms from which the other groups of lower fungi have probably been evolved. It is felt, therefore, that no advantage is gained by sharply separating one series from the others, especially as there is good evidence to show that within the Chytridiaceae-Peronosporaceae series there are many forms which have undoubtedly originated from algae by loss of chlorophyll as a direct result of parasitism. It is considered that these families are to some extent at any rate polyphyletic in origin.

Evidence of the loss of pigment as a result of parasitism is best afforded among the Rhodophyceae. Although in some members of this group the presence of chlorophyll has been demonstrated, it is by no means certain that the green pigment is present in all. In fact there is little reason for considering it necessary. Chlorophyll acts as a photocatalyst, and the red pigment in some cases may function in the same way. It is considered that this red pigment is present in the red algae to assist photosynthesis in deep water. In *Poly-siphonia fastigiata* we have a species which has become an incipient parasite upon *Ascomphyllum nodosum* but as yet it retains its red colour. *Choreocolax* is a genus which is now an obligate parasite on *Callithamnion*. These and other examples indicate the way in which pigmented algae may lose their colouring matter as a result of parasitism. Kruger (79) has shown that *Prototheca Zopfii* and *Chlor-ella protothecoides* represent two closely allied forms, the one containing chlorophyll, while in the other it is absent. It may therefore

be concluded that algae as a result of parasitism have from time to time directly contributed to the fungi.

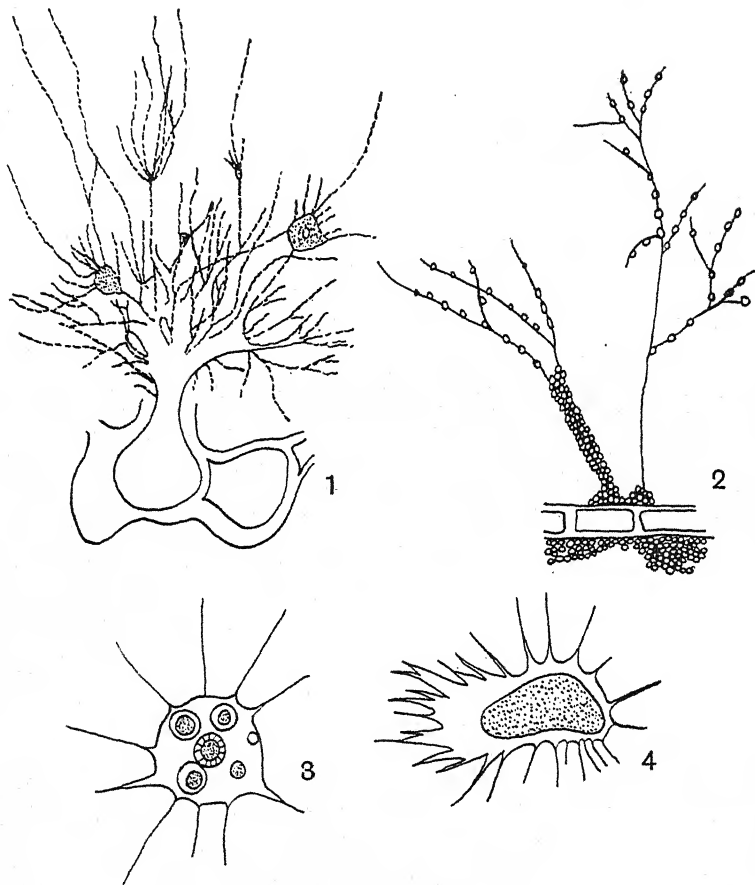
Fritsch(44) has suggested that the algae came from a number of distinct lines passing out from the *Proteomyxa* complex in which chlorophyll has been produced. Similarly it is held that fungi originated from the same complex, from forms which had not succeeded in synthesising a green pigment. If this is the case, it is necessary to look among the *Proteomyxa* for indications of the types from which the fungi may have arisen.

## 2. PROTEOMYXA

According to Lankester the *Proteomyxa* include those members of the Sarcodina (*Proteomyxa* in which reproduction occurs in the amoeboid stage only, and the nucleus frequently gives off fragments which may play a part in the reconstruction after division) which are characterised by the presence of simple pseudopodia. In their life history, the amoeboid soma at some stage becomes converted into a zoosporangium producing zoospores usually with two flagella. Classified according to their amoeboid soma, these organisms belong to the Rhizopoda, which also include the Lobosa or true amoebae, the Foraminifera and the Radiolaria. The two latter groups need not be considered, since they are enclosed in a shell composed of either calcium or strontium. On the other hand, if classified on the formation of biflagellated zoospores, the *Proteomyxa* should belong to the Mastigophora. Cavers(21) is of the opinion that they are derived from the Mastigophora through the Pantostomastigina. Sufficient for the present purpose is the fact that they show in their life history many features which are common to both groups.

In such a form as *Pseudospora volvocis*, whose life history has been described by Robertson(116), both amoeboid and flagellate stages are well developed. When mature, the amoeboid body forms a cyst within which numerous zoospores, each with a single flagellum, are produced. These are liberated, and when they reach a suitable host settle down to an amoeboid existence again. Recent work has shown that a fusion of zoospores may sometimes occur.

In *Polysporella*, described by Zopf(156), the life history differs from that of *Pseudospora volvocis* in the interpolation of secondary cysts. The mature amoeba becomes encysted and then divides into from four to sixteen parts, each of which forms either a fresh resting spore or divides up into four zoospores.

Text-figs. 1-4. *Proteomyxa*

1. *Chlamydomyxa labyrinthuloides*; active phase showing the spindles and particles of algae. (After Archer.)
2. *Labyrinthula vitellina*; a specimen crawling on algae. (After Cienkowski.)
3. *Pseudospora volvocis*; radial form with fine pseudopodia. (After Robertson.)
4. *Vampyrella lateritia*; amoeboid form. (After Hoogenraad.)

In the genus *Vampyrella*, described by Hoogenraad (54), the organism is found in the cells of species of *Mougeotia*, where it penetrates the cell wall and feeds upon the chloroplast. It is amoeboid in shape, with very finely pointed pseudopodia. It may become encysted while still in association with the host filament. The amoeboid soma is really a plasmodium which is formed by the association of amoeboid zoospores.

In the saprophytic genus *Haplococcus*, which lives upon dung, we have a form in which a stalked sporangium is present. In this sporangium four or eight amoeboid zoospores are produced which, on emerging, creep about, probably undergo division and finally coalesce to form a plasmodium which is enclosed within a single cyst or a number of cysts according to its size.

Finally, mention must be made of the genera *Chlamydomyxa* and *Labyrinthula*. *Chlamydomyxa*, of which two species have been described, occurs in Sphagnum bogs in Ireland, Germany and Switzerland. In the active condition it consists of a mass of protoplasm surrounded by a laminated wall of cellulose. In the protoplast are numerous chromatophores containing a green pigment which is considered to be chlorophyll, and also a green or brown pigment which varies in proportion. In the young condition the resting cells are globular and are found lying within the cells of the moss plant. As they grow, they burst out of this confined space and form masses which are frequently visible to the naked eye. They may divide by binary fission, or the protoplast may contract away from the cell wall and divide up into a number of small uninucleate masses which are at first amoeboid but which later round themselves off and form a cell wall. In the active stage, the protoplasm is protruded through the cell wall and forms an anastomosing network of delicate filaments among which fresh protoplasmic masses may be formed later. Sometimes the protoplasm may leave the cell wall and become free in the water. In this active condition, *Chlamydomyxa* is holozoic, catching and ingesting living organisms by means of its pseudopodia and transporting them in the protoplasmic stream to any part of its soma. In the resting stage, it depends upon its pigment for photosynthesis, and lives as a green plant holophytically. Subsequent to a resting period, *Chlamydomyxa* reproduces by the formation of spores which give rise to flagellated zoospores whose further fate has not been followed.

*Labyrinthula vitellina* lives on algae. In its resting stage it consists of a mass of small nucleated cells connected together by a heterogeneous substance. In its active condition it is a plasmodium with long filamentous pseudopodia.

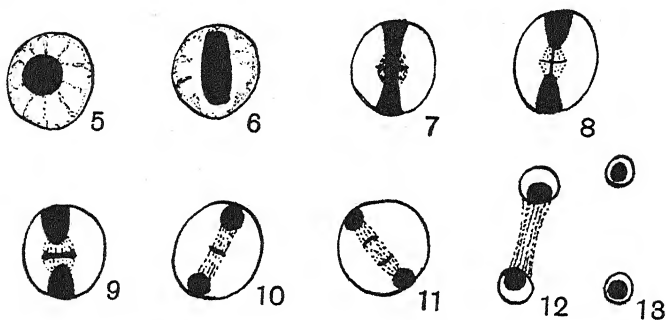
Both these genera exhibit features which may be compared with the Foraminifera and Lobosa on the one hand and with the Mycetozoa, Acrasiales and Chytridiales on the other. They seem most probably to be related to the Foraminifera section of the Rhizopoda through such genera as *Gromia*.

The foregoing types may be taken as examples of the kind of organisms which constitute the Proteomyxa. They are for the most part either saprophytes or parasites with amoeboid and flagellate stages about equally developed. It is important however to remember that the species which remain with us at the present time are in all probability the specialised members of the group, those which by some advantageous evolutionary features have succeeded in adapting themselves to changed conditions and which have enabled them to survive in competition with more advanced forms of life. As a consequence they cannot themselves be considered to typify those primitive forms from which the lower fungi have originated. They give us indications of what those organisms were like, but they are not themselves those forms.

Turning to the question of cytology, we find that very few of these simple organisms have been investigated. Alexeieff(2) has shown that it is possible to distinguish some ten different forms of nuclear division, all of which are separable from true mitosis. The examples selected are drawn largely from the lower members of the Protozoa. Of these types he distinguishes five main forms which he calls Promitosis, Haplomitosis, Mesomitosis, Paramitosis and Panmitosis. The fundamental difference between these and true mitosis lies in the fact that no chromosomes are differentiated, although a method exists for equally dividing the chromatin within the nucleus. They are however quite distinct from amitosis, in which the nucleus fragments into a variable number of parts, in which no provision is made for the chromatin elements to have any genetical continuity.

In the primitive fungi so far as is known mitosis is the only method of division in any of the groups, excepting the Plasmodiophorales. It is generally held that the nucleus is the most conservative part of the cell of any organism, and we may therefore conclude that where a type of nuclear division simpler than mitosis is found, such a group will be more primitive than one which undergoes mitosis. It is probable that protomitotic nuclear divisions represent various experimental phases in the evolution of a typical mitosis, and do not represent a retrogressive or degenerate series.

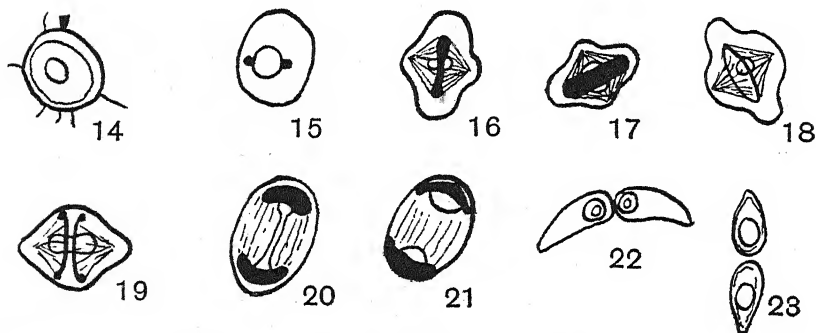
We find various stages of evolution occurring in different members of the Lobosa. Alexeieff finds that promitosis exists in the *Limax* section of the Amoeboae, protomitosis in *Amoeba diploidea*, paramitosis in amoebae parasitic in vertebrates, and panmitosis in several other species, while at the same time a perfectly typical mitosis



Text-figs. 5-13. Protomitosis in *Amoeba mucicola*

5. Resting nucleus with central karyosome.
6. Elongation of the karyosome.
7. Formation of chromatin ring around the karyosome.
8. Division of the karyosome before the chromatin.
9. Thickening of chromatin ring.
10. Reformation of the daughter karyosomes.
11. Split in the chromatin ring.
12. Re-formation of the daughter nuclei.
13. Daughter nuclei after division.

(All after Chatton.)



Text-figs. 14-23. Protomitosis in *Ligniera junci*

14. Resting nucleus.
15. Beginning of the chromatin ring around the karyosome.
16. Chromatin ring with central karyosome and spindle.
17. Thickening of chromatin ring.
18. A view showing the spherical nature of ring of chromatin.
19. Splitting of the chromatin ring.
20. Complete division of chromatin and elongation of the karyosome.
21. Division of chromatin and karyosome into two.
22. Division of the nuclear membrane into two.
23. Re-formation of daughter nuclei.

has been described by Dobell(38) and others(1, 45) in various species.

In 1910 Chatton(22) published a cytological account of the nuclear divisions in *Amoeba mucicola*. The cytology described there agrees in all essentials with Alexeieff's type of promitosis. I have compared elsewhere(29) in detail the close similarity between this form of protomitosis and that which has been repeatedly shown to occur in all the members of the Plasmodiophorales of which the cytology has been studied. Briefly, in both, the chromatin in the resting nucleus is aggregated around the periphery in close association with the nuclear membrane. In the centre of the nucleus is the karyosome. In division, the chromatin becomes aggregated in a ring around the karyosome suspended by a more or less definite spindle. Both the chromatin ring and the karyosome now split and the halves pass to the poles. The chromatin then becomes reformed around the karyosome. During the whole process the nuclear membrane remains intact, and at the end of the division invaginates to form the membranes of the daughter nuclei. In this procedure we see an incipient method of division of chromatin into equal halves, but no distinct chromosomes ever become differentiated. Such a condition must be considered primitive. *Amoeba mucicola* is not a very specialised member of the Lobosa in other respects, so that such a conclusion seems justified. The Plasmodiophorales on the other hand exhibit certain specialised features. Although during the growth of the soma all the nuclei divide by the form of protomitosis described above, in the formation of spores a more or less typical meiosis occurs in which definite chromosomes are differentiated.

### 3. PLASMODIOPHORALES

The plasmodium of the Plasmodiophorales may with reasonable certainty be compared with the soma of an amoeba. In *Diplophyrus stercorea* and in the genus *Gymnococcus* among the Proteomyxa, amoeboid individuals aggregate together to form plasmodia. It is considered that in this way the soma of the Plasmodiophorales has originated by the aggregation of amoebae. These amoebae still exhibit a primitive type of nuclear division—a protomitosis similar to what is still found in the true Amoebae as represented by *Amoeba mucicola*. At the same time, some method of sexual reproduction with a nuclear fusion had been evolved, with the result that a method of chromatin reduction was necessary. We know very little about the gradual evolution of meiosis, but inasmuch as fusion of nuclei



meant doubling of the chromatin we must conclude that a reduction division was present in the simpler forms. The Plasmodiophorales apparently found it difficult to reconcile protomitosis with meiosis, and consequently we find an intermediate stage which is generally termed the akaryote, in which all the chromatin of the nuclei is extruded into the cytoplasm and when reformed has the general appearance of a typical meiotic stage. Such a meiosis has been demonstrated in some of the higher amoebae.

We see then that in the Plasmodiophorales as we now know them we have forms which while retaining some primitive features have in other respects progressed far on the road of specialisation. At the same time they do not appear to lead anywhere. All the species known are very remarkably similar even in the smaller details and they show among themselves no definite sequence of type. In the presence of a plasmodium and in the character of a somatic nuclear division it is possible to relate them closely with the amoebae through a form like *Amoeba mucicola*.

We may conclude therefore that starting from a primitive colourless member of the Proteomyxa without any true mitosis we have a series of forms which give rise to the Lobosa. These, at first, were simple uninucleate organisms devoid of any sexual reproduction. Later, by the aggregation of many individuals, a plasmodium was formed, though it still retained the same type of nuclear division. Then nuclear division became more complex, and both mitosis and meiosis were evolved, possibly in direct relation to the appearance of sexual reproduction. Somewhere between the entire abolition of a protomitosis and the final adoption of mitosis the Plasmodiophorales became differentiated. A considerable number of forms must be missing from this series. In the first place, all the existing members are parasites on Phanerogams except one, which is found in insects. It seems probable that the intervening types may still exist in some of the higher orders of Cryptogams, which would help to bridge the gap between a holozoic amoeba and a parasitic member of the Plasmodiophoraceae living in the tissues of Phanerogams.

The Mycetozoa in many respects resemble the Plasmodiophorales. They may possibly be looked upon as a parallel series which instead of becoming parasites developed along saprophytic lines. Their life cycle is on the whole very satisfactorily understood. Recent work by Skupienski<sup>(127)</sup> and also by Wilson and Cadman<sup>(153)</sup> has suggested some interesting points on the question of the nuclear behaviour in the development of a plasmodium. The method of

plasmodium formation differs in some respects from that found in the Plasmodiophorales. In the former it is the product of a number of separate uni- or multi-nucleated amoebae which become aggregated together to form a single soma. In the Plasmodiophorales as far as we know the whole plasmodium is the product of a single uni-nucleate amoeba. This difference however is not so important as it may appear at first sight. In the Mycetozoa the plasmodium swarms over a free surface which offers admirable facilities for collecting up any stray amoebae and incorporating them into its own body; the association of different amoebae is free from any obstacles, and in fact might naturally be expected when amoebae all produced from a single mass of swarm spores are moving about in close association. In the Plasmodiophorales the first act of the swarm spore is to obtain entry to the host tissue, and it is only a matter of chance that more than one swarm spore finds its way into the same cell. Moreover, the supply of food in a single host cell is necessarily limited, with the result that the association of two amoebae in the same cell would be a disadvantage rather than an advantage. Furthermore, the size of the mature plasmodium is limited, firstly by the size of the host cell and secondly by the quantity of available food substances. It is considered therefore that there is no fundamental difference between the plasmodium of the Mycetozoa and the multi-nucleated amoeba of the Plasmodiophorales. The points of difference are the direct result of their particular habitat rather than of morphology.

The chief difference between the plasmodium of the Mycetozoa and that of the Plasmodiophorales is to be found in their method of nuclear division. In the Plasmodiophorales we have seen that this is effected by a definite type of protomitosis, whereas in the Mycetozoa all the nuclei divide by mitosis. We are therefore justified in concluding that the plasmodium of the Mycetozoa is more advanced than that of the Plasmodiophorales, though it does show many points of affinity with the Amoebae on the one hand and with the Plasmodiophorales on the other. The view adopted here is that Mycetozoa diverged from the Amoeba series at a higher point than did the Plasmodiophorales, at a stage, that is to say, when typical mitosis had already been evolved. In both these groups we find that specialised methods have been introduced to provide for spore formation. Again, the Mycetozoa are more specialised than most members of the Plasmodiophorales, since the former have evolved light spores with a very resistant coat suitable for wind distribution. The Plasmodiophorales, as typified by the genus *Ligniera*, have still

retained zoospores as a means of dissemination though they too have thick-walled spores, but the balance of probability is that these are associated with protection during the winter rather than that they are remnants of a pre-existing ancestor which had evolved thick-walled spores for aerial distribution prior to becoming parasitic.

In the view adopted here it is held that the Plasmodiophorales and the Mycetozoa have originated from the Lobosa, this latter group being represented evolutionarily by a series of forms starting with the *Proteomyxa* and developing along the lines of true amoebae. During this process, successive stages in the evolution of mitosis can be traced. On the type of nuclear division presented in the vegetative phase of the life history, it is held that the Plasmodiophorales diverge from this series at a more distant point than did the Mycetozoa.

These conclusions are made clear by the schematic representation set out in Diagram I:

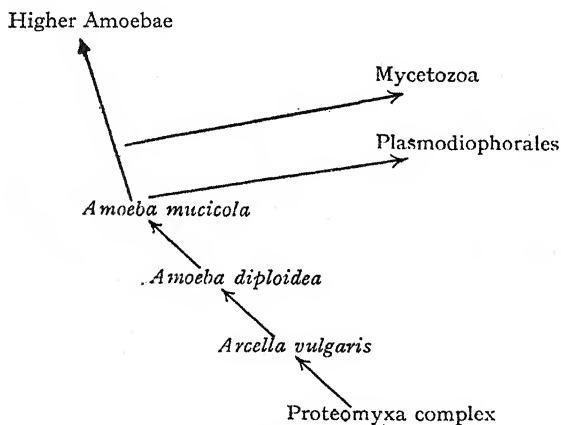


Diagram I

Turning now to a consideration of the relationships of the families and genera grouped respectively in the Plasmodiophorales and Mycetozoa, we find remarkable uniformity present. The features upon which classification is chiefly based are the shape and general character of the spore masses. In *Plasmodiophora brassicae* (155) the spores are free and not apparently arranged in any definite system. In *Spongospora subterranea* (105) they are aggregated in what is generally referred to as "spongy masses." These masses do not

show any constant characteristic shape. In *Sorosphaera veronicae* (15) they are in hollow spheres while in *Sorodiscus callitrichis* (154) they are in flattened spheres and ellipsoids. In *Tetramyxa parasitica* (92) the spores are found in tetrads. In *Sporomyxa Scauri* (83) the spores are not arranged in any system, and the individual spores are ellipsoidal. In the genus *Ligniera* (26) again, there is no characteristic system exhibited, although in some cases there is evidence of their being grouped in hollow spheres. Of the remaining genera very little is known; reasons have been given elsewhere (26) for considering the genera *Clathrosorus* (41) and *Ostenfeldiella* (40) of doubtful validity. In any event, the spore system is closely similar to that of *Spongospora*. In *Molliardia triglochinis* (92) no spores have been found, and as Schwartz (122) points out, when these become known, this species may be transferred to another existing genus. In *Plasmodiophora tabaci* (66, 67) and the other doubtful species which have been described in that genus, the spore system, where it has been observed, is similar to what is found in *Plasmodiophora brassicae*.

Although schizonts have been described by Maire and Tison (92) in *Molliardia triglochinis*, in no genus except *Ligniera* has the existence of both spores and zoospores been demonstrated. Moreover, in not causing hypertrophy of the host tissue *Ligniera* stands apart from the other genera. It has previously been shown (27) that factors associated with the habitat react differently upon the various members of the Plasmodiophorales, and therefore are of little value in phylogeny. These factors are undoubtedly related to the particular environment in which the parasite lives and to which it has become adapted in the course of its evolution.

It has already been pointed out that cytological characteristics do not contribute any information regarding the phylogeny of the group. Basing the phylogeny therefore upon such characters as we possess, it may be said that an organised system of spore groups is probably more specialised than one in which the spores are not arranged in any system. On these grounds it is held that the genus *Plasmodiophora* is primitive, and may be taken as the starting point of a series through *Spongospora* to *Sorodiscus* and *Sorosphaera* and ending with *Tetramyxa*. In not causing hypertrophy and in forming zoospores, *Ligniera* stands upon a side line from *Plasmodiophora*. *Sporomyxa* found in the bodies of insects also differs considerably in the formation of isolated ellipsoidal spores, and has probably originated from a form more primitive than *Plasmodiophora*, but shows little relationship to *Ligniera*.

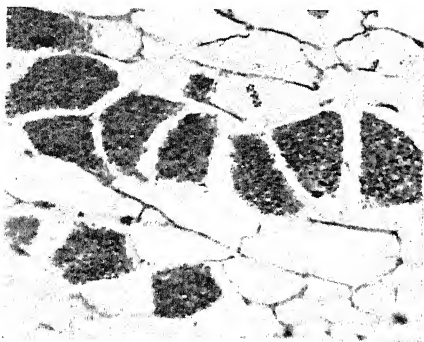


Fig. 1. *Plasmodiophora brassicae* Woron.

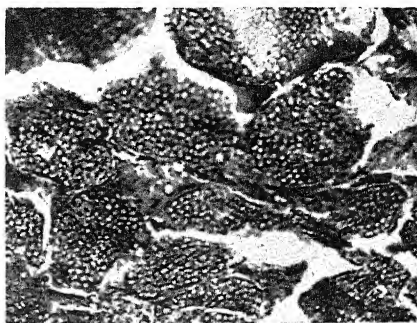


Fig. 2. *Spongospora subterranea* (Wallroth.) Johnson.

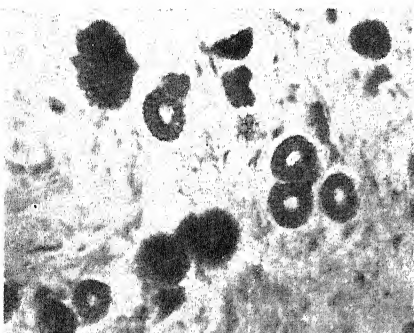


Fig. 3. *Sorosphaera veronicae* Schröt.



Fig. 4. *Sorosphaera radiale* sp. nov.

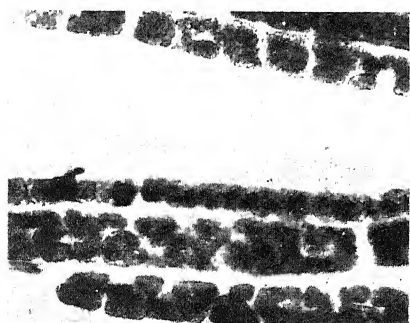


Fig. 5. *Ligniera junci* (Schw.) M. and T.  
*sensulate*.

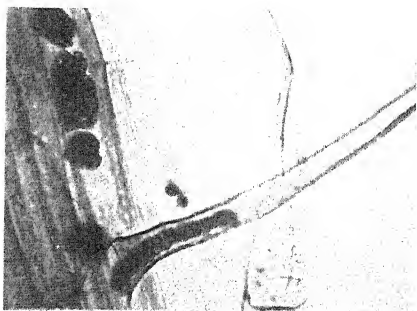
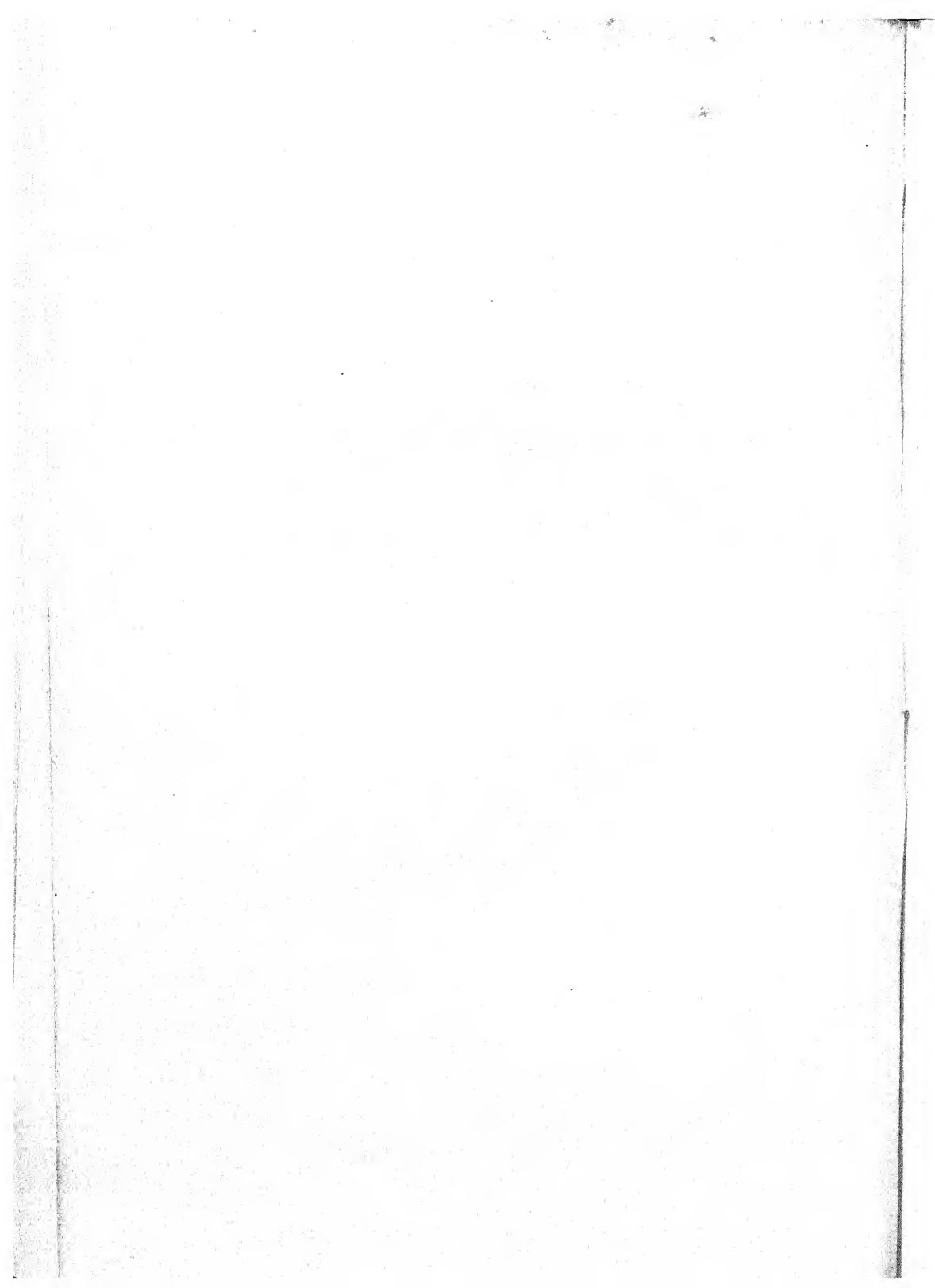


Fig. 6. *Ligniera verrucosa* Maire and Tison.

Photographs of the spore masses of Plasmodiophoraceae.



These conclusions are set out in tabular form in Diagram II. This phylogenetic series only represents the probable sequence of stages in the evolution of the group, for it must be remembered that only a few isolated end lines now remain known to us.

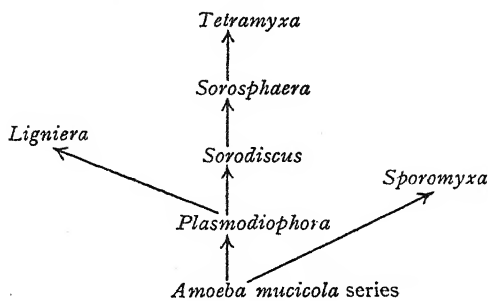


Diagram II

#### 4. MYCETOZOA

The Mycetozoa, like the Plasmodiophorales, are a very united group with comparatively few indications of a phylogenetic series. Of the two groups Exosporeae and Endosporeae, into which they are divided, the Exosporeae are represented by the single species *Ceratiomyxa fruticulosa*, which has been studied by various workers (60). Its life history is known beyond all doubt. In many respects it stands apart from the whole of the Endosporeae, and its chief difference from that group lies in the fact that the spore, instead of containing a single nucleus as is characteristic of the Endosporeae and also of the Plasmodiophorales, possesses four nuclei. After a brief period of amoeboid existence, all four nuclei divide mitotically and the whole cell divides up into eight parts. Each acquires a flagellum and swims off as an independent swarm spore. *Ceratiomyxa* also differs from the rest of the Mycetozoa in the formation of separate spores on distinct sporophores. In fact it seems probable that *Ceratiomyxa fruticulosa* diverged from the Mycetozoa-Proteomyxa series at a comparatively early stage. The method of producing spores suggests another and not very successful method of dealing with propagation. In fact in the first place the spores are ill protected and both they and the sporophores are dependent upon not too dry conditions. If these persist the whole structure becomes desiccated and dries up. On the other hand very damp weather entirely prevents the distribution of the spores by wind. The protection of

the plasmodium within the tissues of the host is probably the one feature which has made the survival of this species possible.

Turning now to the Endosporeae we find a very uniform group, the species of which differ from one another only in minor points. In fact the only reasonable character upon which phylogeny can be based is upon the presence or absence of a capillitium. In the Calcarineae the sporangium is provided with granules of carbonate of lime which probably represent a method of disposing of the by-products of metabolism. In the Stemonitaceae we find this lime absent though the structure of the capillitium is somewhat similar. In the Lamprosporaes we find the capillitium either represented by a system of uniform or sculptured rods or threads, or absent altogether. It is difficult to suggest any reasonable phylogeny among the families which make up the Endosporeae. We know practically nothing of the past history of the group nor the tendencies which prompted either their saprophytic habit or the development of aerial sporangia with spores adapted for wind distribution. All that we can say is that these latter features are not generally associated with primitive organisms, and that therefore the balance of evidence is in favour of a long series of ancestral forms, now extinct, between the primitive amoeba and the present-day Mycetozoa. Capillitium formation is probably specialised and not a primitive feature, and we may be therefore justified in concluding that those members in

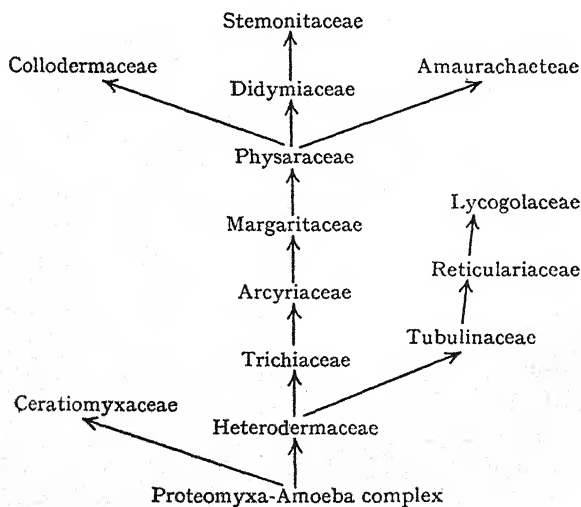


Diagram III



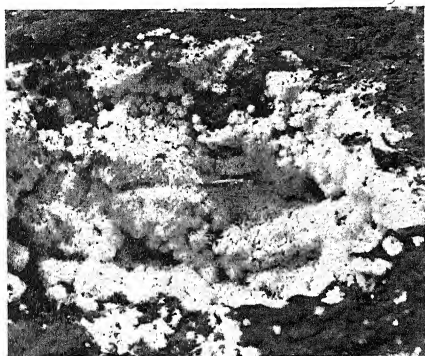


Fig. 1. *Ceratiomyxa fruticulosa* Macbride.

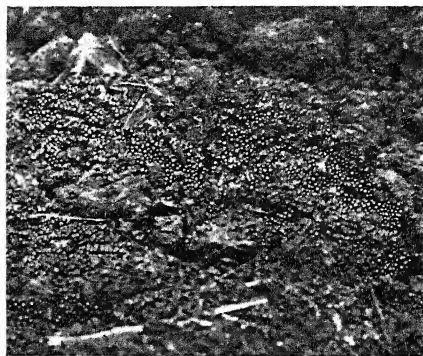


Fig. 2. *Cribraria argillacea* Pers.

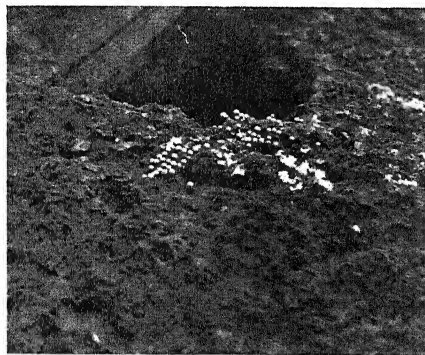


Fig. 3. *Trichia varia* Pers.

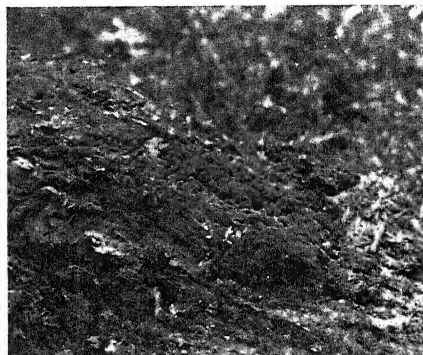


Fig. 4. *Arcyria denudata* Wettstein.

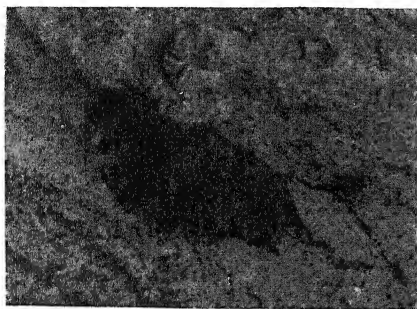


Fig. 5. *Reticularia Lycoperdon* Bull.

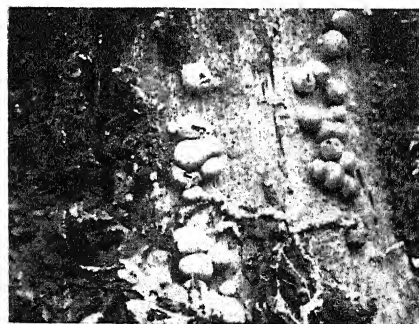
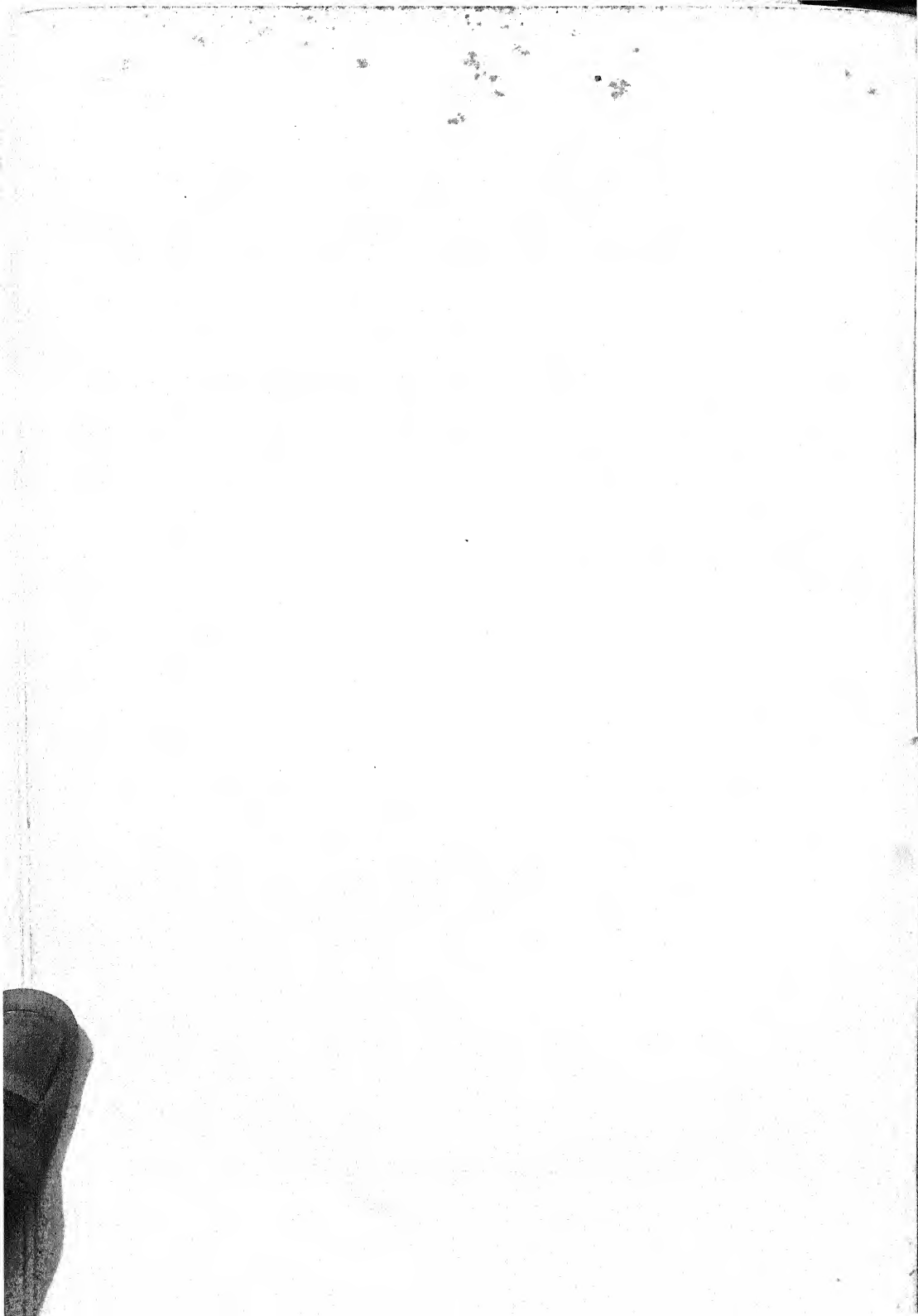


Fig. 6. *Lycogola epidendrum* Fr.

Habit photographs of Mycetozoa.



which such a structure is absent or only slightly developed are simpler than those in which it is highly specialised.

The schematic representation on p. 246 is put forward as a suggestion to illustrate the general tendencies within the Endosporeae.

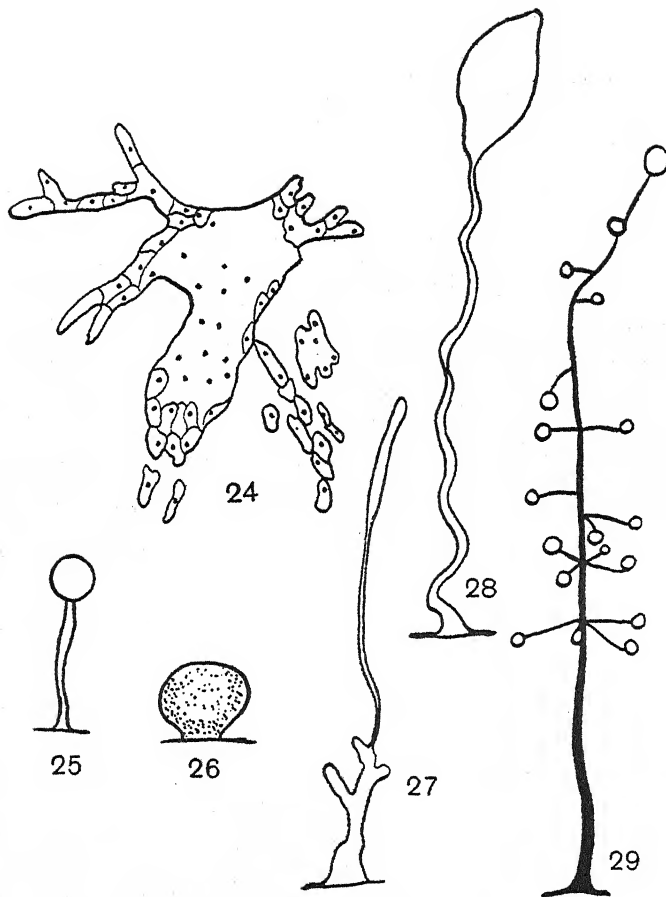
Such a scheme as is represented in Diagram III suggests a monophyletic origin for the whole of the Endosporeae. Aethalia have appeared several times in the course of evolution but almost undoubtedly were end-lines, the result of over-specialisation and large spore production. The genus *Tubifera* suggests a way in which a transition from separate sporangia to complete aethalia was effected.

### 5. ACRASIALES

We now come to the third division of the Archimycetes, the Acrasiales. They are all small saprophytic forms living upon dead wood or dung. Only a few genera have been described, and very little is known about their cytology. De Bary<sup>(13)</sup> and van Tieghem<sup>(134)</sup> consider them to be related to the Mycetozoa, and suggest a common ancestor. De Bary thought that the plasmodium of the Acrasiales was derived from that of the Mycetozoa. He suggested two lines of evolution within the group, the one progressive, giving rise to the genus *Polysphondylium*, and the other retrogressive, producing forms like *Sappinia*. Between these two series he places *Guttulina* as a possible starting point of the two divergent lines.

Harper<sup>(51)</sup> agrees with de Bary in his ancestry of the Acrasiales but points out a progressive series to the more specialised Mycetozoa. He considers that the capillitium found in the higher Mycetozoa was a secondarily evolved structure associated with spore discharge and not directly homologous with any organ pre-existing in the Acrasiales.

If we accept Harper's view, it follows that the plasmodium of the Mycetozoa has been evolved from the pseudoplasmodium of the Acrasiales by the coalescence of independent individuals to form a single united soma in which the amoebae lose all trace of individuality. Zopf<sup>(156)</sup> has pointed out that the pseudoplasmodium of the Acrasiales is not homologous in many ways with the true plasmodium. The nuclei of the plasmodium increase in number both during the vegetative phase and again just prior to reproduction, whereas in the Acrasiales there is no division of the myxamoebae after their aggregation to form a pseudoplasmodium.



Text-figs. 24-29. Acrasiales

- 24. *Polysphondylium violaceum*; pseudoplasmodium.
- 25. *Dictyostelium brevicaulis*; fructification.
- 26. *Guttulinopsis vulgaris*; sessile sorus.
- 27. *Polysphondylium violaceum*; young fructification.
- 28. *Dictyostelium violaceum*; development of pseudoplasmodium.
- 29. *Polysphondylium violaceum*; mature fructification.

(All after Olive.)

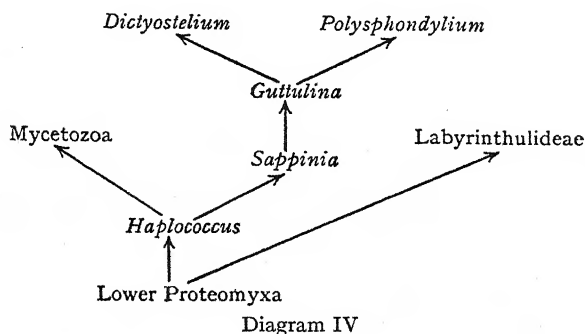
Olive(104) considers that the absence of swarm spores in the life history of the Acrasiales is a point of considerable importance, and moreover the morphology of the plasmodium and the pseudoplasmodium cannot be compared except through the Amoebae. Zopf thought there was a relationship between the Labyrinthulideae and

the Acrasiales. He suggests that the net plasmodium of the former is an intermediate condition between the Acrasiales and the Mycetozoa. It is important however to note that that part of the life cycle of the Labyrinthulideae most comparable with the Acrasiales is the heaping up of individuals prior to their encystment in fructifying masses. The peculiar motion of the Labyrinthulideae on the other hand is very different from that found either in the Acrasiales or Mycetozoa.

Among the Acrasiales we find that *Sappinia* is most closely allied to the true Amoebae. In many respects it is a true member of the Lobosa, and suggests at once a direct phylogeny with the Proteomyxa. *Guttulina* represents a more advanced condition, and the Dictyostelaceae the most specialised. It is therefore with either *Sappinia* or *Guttulina* that we should expect to relate members of the Proteomyxa. Mention has already been made of the genus *Haplococcus*. Unfortunately, comparatively little is known about this organism. The fact that the sporangium possesses amoeboid spores suggests a relationship both with the Acrasiales and with the Mycetozoa. The vegetative soma is a plasmodium, though very little is known about its method of growth or of the fate of the amoeboid swarm spores. Since *Haplococcus* is a saprophyte, it would act as a very valuable link in a series of saprophytic sporangial forms relating the Proteomyxa with the Mycetozoa and the Acrasiales. In the formation of an aerial sporangium we have a very good indication of the way in which the fruiting bodies of the Mycetozoa were evolved: presumably these first had amoeboid spores which later became adapted for wind distribution. Diagram IV represents the conclusions which have been arrived at regarding the phylogeny of the Acrasiales.

Reference to Diagram I will show that in the scheme set out there it was suggested that the Mycetozoa originated from a Lobosa series which had already evolved through mitosis, yet as suggested in Diagram IV it may be argued that the Mycetozoa are derived from the Proteomyxa directly through *Haplococcus*. These two schemes are not really contradictory, but indicate that the Amoebae as typified by *Amoeba mucicola* belong to the Proteomyxa complex. They are forms not yet sufficiently differentiated to justify their being considered as true Amoebae, yet at the same time already show a decided tendency towards the Lobosa. It is considered that *Haplococcus* was evolved from this series of types, and represents an amoeba in the wide sense which had produced a very primitive

sporangium as an additional structure. In this way the two series fall into alignment, both emerging from the same *Proteomyxa* complex, but diverging almost before leaving the *Proteomyxa* slightly later than the *Plasmodiophorales*.



## 6. CHYTRIDIALES

We now come to the fourth and by far the most obscure group which is included in the Archimycetes—the Chytridiales. Within this group we have a great assemblage of forms which differ widely among themselves both in structure and methods of reproduction, and also from the orders we have so far considered. We find on the one hand very simple structures consisting of little more than a single zoosporangium, and possessing no differentiation between vegetative and reproductive phases, to species with well-marked sex organs and a well-developed mycelium on the other. In addition to these there are isolated forms or groups which in all respects except for the absence of chlorophyll resemble the algae. It is in this diverse assemblage that one must attempt to trace phylogeny.

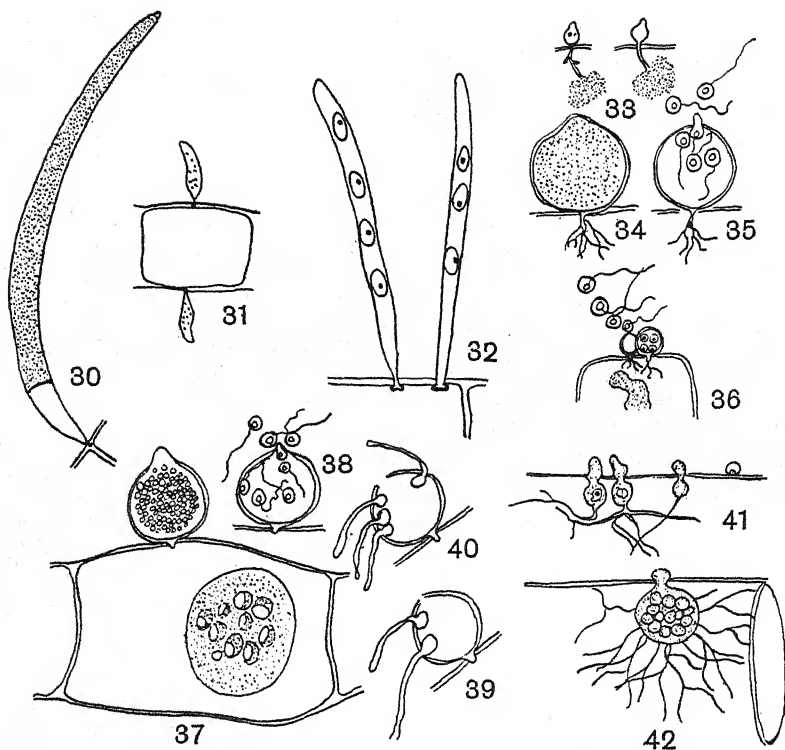
De Bary<sup>(14)</sup> and his co-workers have related the Chytridiales with the Peronosporales or Saprolegniales through the Pythiaceae and Ancylistaceae as a reduction series brought about as a direct result of parasitism. Workers previous to de Bary moreover had grouped the Saprolegniaceae in the genus *Conserva*. Other workers pointed to the close resemblance between *Pythium* and *Vaucheria*.

In 1901 Dangeard suggested that the Chytridiales might have originated from the zoosporic Monadineae. Atkinson<sup>(9)</sup> doubted whether Dangeard's basal type was the correct one, but came to the conclusion that the series of forms found in the Chytridiales is more easily explained as a progressive series from the Protozoa than

as a retrogressive one from the Peronosporaceae. Working on this hypothesis he comes to the conclusion that it is in the Olpidiaceae that we find forms most closely allied to the original primitive protozoan ancestor. If this is the case, we have to consider how the much more algal forms like *Harpochytrium* originated. Atkinson is of the opinion that they were produced through lack of penetrative power on the part of the zoospores which, instead of passing into the tissues of the host, started to do so but went no farther, and formed their zoosporangia outside instead of inside the host cell. *Harpochytrium* on the other hand shows considerable morphological similarity to the Green Algae, and more particularly with forms like *Characium*. Butler (18) considers that the number of flagella is an important feature in the classification of the Chytridiales. If this is true, then *Characium*, with two flagella to its zoospores, is sharply separated from *Harpochytrium* where there is only one.

The genus *Rhizophidium*, however, contributes many forms which give a clue to the phylogeny of these genera. In *Rhizophidium brevipes* and *Rhizophidium globosum* the zoosporangium is formed outside the host cell. In *Rhizophidium brevipes*, only an attaching disc is present, while in *Rhizophidium globosum* a series of short hyphae are formed which ramify within the tissues of the host. Finally, in *Entophlyctis bulligera*, described by Zopf (156), although the remains of a zoosporangium are indicated outside the host cells the functional zoosporangium is organised within the host tissues, and moreover there is a more or less strongly developed mycelium which may extend into adjacent cells. It is possible in this way to see how such forms as *Harpochytrium* and *Rhabdium* may have become endoparasites, but it does not explain the origin of a *Harpochytrium* type.

Among the Protozoa, it is not difficult to find forms in which reproduction is effected by zoospores and whose zoosporangia at the same time may be compared with *Harpochytrium*. Some of the genera figured by Stein (129) may be taken as examples. The type organisation consisting of a short epiphytic sporangium may quite reasonably have had a common ancestry but that does not by any means imply that that ancestor was a green alga. It is more probable that in this case we have an example of parallel evolution resulting in the perfection of a complex organism from a small zoospore unable to penetrate the host tissue. Again, the chlorophyll in *Characium* may have come directly from a type in which no green pigment was present.



Text-figs. 30-42. Chytridiales

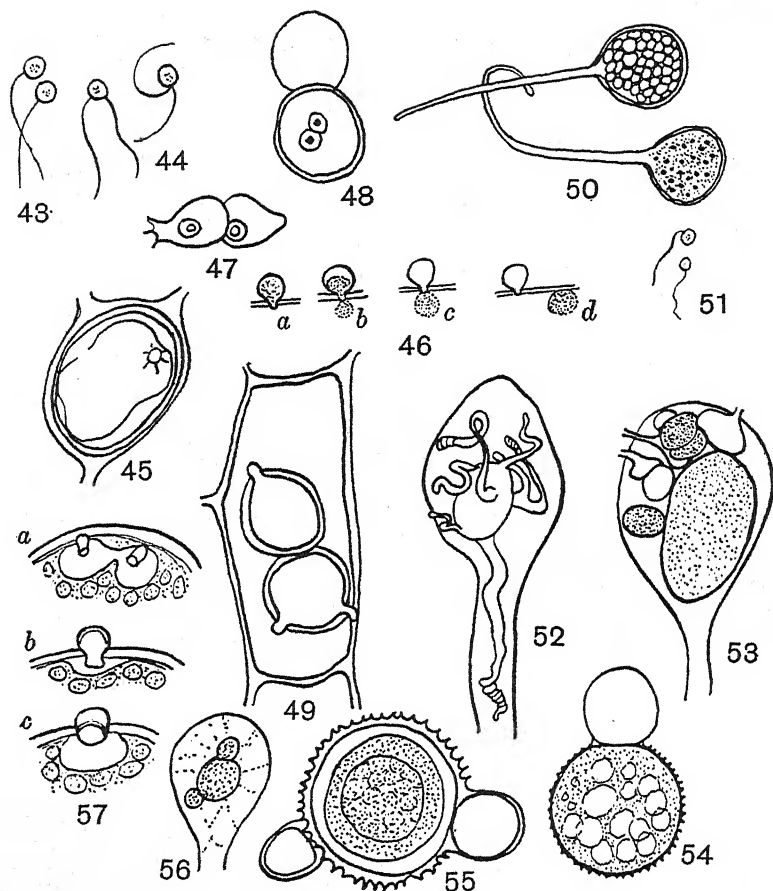
30. *Harpochytrium hedenii*; zoosporangium. (After Atkinson.)
31. *Harpochytrium hedenii*; two zoosporangia. (After Atkinson.)
32. *Rhabdium acutum*; zoosporangia. (After Dangeard.)
33. *Rhizophidium brevipes*; zoospores entering host cell. (After Atkinson.)
34. *Rhizophidium brevipes*; mature zoosporangium with rhizoids. (After Atkinson.)
35. *Rhizophidium brevipes*; escape of zoospores. (After Atkinson.)
36. *Rhizophidium brevipes*; zoospores becoming attached to host wall. (After Atkinson.)
37. *Rhizophidium globosum*; zoosporangium. (After Atkinson.)
38. *Rhizophidium globosum*; escaping zoospores. (After Atkinson.)
39. *Rhizophidium globosum*; two zoospores attempting to escape through germ tubes through the wall of the zoosporangium. (After Atkinson.)
40. *Rhizophidium globosum*; later stage showing their retreat into the zoosporangium and fresh escape through germ tubes. (After Atkinson.)
41. *Entophlyctis bulligera*; young plant showing the zoospores on the outside forming a thallus. (After Zopf.)
42. *Entophlyctis bulligera*; mature plant. (After Zopf.)



It seems reasonable to consider that we have two distinct series, the one in which the zoosporangium was formed from zoospores which had the power of penetrating the tissues of their host, and the other in which the zoospores at first merely attached themselves to their host and developed without complete penetration. In the first series we find the Olpidiaceae and in the second the Rhizidiaceae.

We will consider the Olpidiaceae first. In the genus *Sphaerita* (34) the wall of the zoosporangium disorganises when the zoospores are mature, and they escape into the host tissue. In the genus *Olpidium*, as typified by *Olpidium brassicae*, large exit tubes are formed to facilitate this process. In *Olpidium viciae* described by Kusano (81), and in *Olpidium radicale* which has been recently described elsewhere (123), we find a transitional condition. In these forms two types of zoosporangia are produced, the one with a thick wall and the other with a thin. Exit tubes are developed in the thick-walled zoosporangia, but they are absent in the others. Moreover, in both species these exit tubes are poorly developed and frequently do not penetrate the wall of the host cell. When we pass from the genus *Olpidium* to *Olpidiopsis* (11) much more complex exit tubes are developed. One might therefore trace a series from *Olpidium* to *Olpidiopsis* from the development of exit tubes alone. There is however further evidence. Kusano has shown that in *Olpidium viciae* the zoospores frequently conjugate in pairs outside the host plant, and he is of the opinion that the product of this zygote is a thick-walled zoosporangium. In *Olpidium radicale* it has been shown that conjugation occurs between zoospores after they have entered the host tissue, and then only if they are in the same cell. Fusion may be delayed for some time with the result that though in some cases it is isogamous, very frequently zoospores of different sizes come into contact. The result of this fusion is the thick-walled zoosporangium. Barrett (11) has shown in *Olpidiopsis* that an antheridium and an oogonium are formed, the two structures being quite distinct in size and shape. It is held that these factors are sufficient to demonstrate an evolutionary series from *Sphaerita* through *Olpidium* to *Olpidiopsis*.

The Synchytriaceae stand apart from these forms. True, they have both thick- and thin-walled zoosporangia, but the complicated mechanism of inhibition of water associated with the bursting and the general way in which the zoospore is formed, such as has been described by Curtis and others (33), suggest that they are an offshoot



Text-figs. 43-57. Olpidiaceae

43. *Olpidium viciae*; zoospores. (After Kusano.)
44. *Olpidium viciae*; conjugation of zoospores. (After Kusano.)
45. *Olpidium radicale*; zoosporangium showing the opening.
46. *Olpidium viciae*; a-d, germination of the zygote. Stages in the entrance into the host cell. (After Kusano.)
47. *Olpidium radicale*; conjugation of zoospores after entering host cell.
48. *Olpidium radicale*; formation of zygote by copulation of the gametes within the host cell.
49. *Olpidium radicale*; empty zoosporangia showing the exit tubes.
50. *Olpidium brassicae*; zoosporangia showing the exit tubes.
51. *Olpidium brassicae*; zoospores.
52. *Olpidiopsis vexans*; showing the exit tubes in a hypha of *Saprolegnia*. (After Barrett.)
53. *Olpidiopsis vexans*; showing discharged and undischarged zoosporangia. (After Barrett.)
54. *Olpidiopsis vexans*; nearly mature oospore. (After Barrett.)
55. *Olpidiopsis luxurians*; mature oospore with two empty antheridia. (After Barrett.)
56. *Olpidiopsis vexans*; oogonium and two antheridia. (After Barrett.)
57. *Olpidium viciae*; a-c, stages in the bursting of the zoosporangium. (After Kusano.)

from the *Olpidium* series. Both the Olpidiaceae and the Synchytriaceae show many points of similarity in their nuclear behaviour. In both, considerable discharge of chromatin is associated with nuclear division, with the result that Griggs (47) among others has considered them to be amitotic. This feature is undoubted evidence of affinity. Moreover we find the same faculty of zoospores to function as gametes in the genus *Synchytrium* as has been described in *Olpidium viciae*.

Recently it has become customary to include the family Woroninaceae which contains the genera *Woronina*, *Woroninella*, *Rosella* and *Pleolpidium*. Very little is known about these, but they seem to fall more or less in line with the genus *Olpidiopsis*, except that two flagella instead of one has been described by some workers. When more is known about them they will probably be more closely related to *Olpidiopsis* and the *Olpidium* series. Griggs' genus *Monochytrium* (48) has not been fully described but in a general way one may argue with Atkinson (10) that it should be placed in the Olpidiaceae.

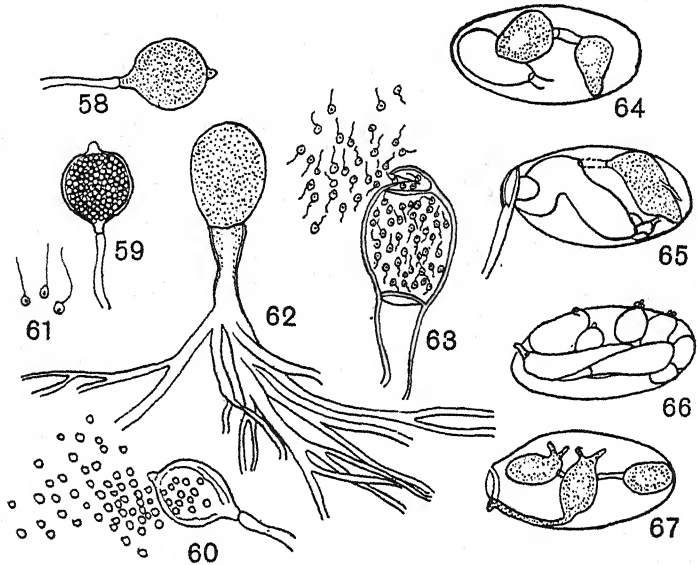
Starting, therefore, from a simple form like *Sphaerita* we can trace a series which finally gives us a type without any appreciable mycelium, but nevertheless with well-defined sex organs.

Through the *Harpochytrium* series we can trace a sequence starting from an epiphytic form in which the apex of the zoospore functions merely as a holdfast as in *Harpochytrium* itself, to *Rhabdium* in which the holdfast completely penetrates the host tissue but still forms its zoosporangium outside, and still further to such forms as *Rhizophidium globosum* in which the holdfast becomes modified as a rhizoidal structure and thence to *Entophlyctis bulligera* in which the zoosporangium is found inside the host cell. It is important to note that all these forms possess zoospores with a single flagellum.

Atkinson has shown that certain species of *Rhizophidium* such as *Rhizophidium brevipes* and *Rhizophidium sphaerocarpum* may be related to the Cladochytridiaceae. When the zoospores are unable to enter the host cell they develop short mycelial tubes. This condition is very similar to that which is found in the genus *Catenaria*. In that genus the mycelium which is formed by the zoospores at once swells up and produces a zoosporangium from which secondary sporangia are formed. Butler and Buckley (20) and Butler (19), who have studied *Catenaria anguillulae* recently, and have shown the way in which the zoospores develop into a fresh zoosporangium while penetrating the wall of the ova of *Fasciola hepatica*, have also demon-

strated that the zoospores possess a single flagellum. These facts help to relate *Catenaria* with the genus *Rhizophidium*.

According to Schroter(119), the Chytridiales were divided into six families, among which he included a group to which he gave the name of Oochytridiaceae. In it he placed four genera, *Polyphagus*, *Diplophysa*, *Urophlyctis* and *Zygochytrium*. Most recent writers



Text-figs. 58-67. Chytridiales

- 58. *Hyphochytrium infestans*; young zoosporangium. (After Zopf.)
- 59. *Hyphochytrium infestans*; zoosporangium forming zoospores. (After Zopf.)
- 60. *Hyphochytrium infestans*; escape of zoospores. (After Zopf.)
- 61. *Hyphochytrium infestans*; zoospores. (After Zopf.)
- 62. *Macrochytrium botrydioides*; mature plant. (After von Minden.)
- 63. *Macrochytrium botrydioides*; escape of zoospores. (After von Minden.)
- 64-67. *Catenaria anguillulae*; sporangia developing in the eggs of *Fasciola hepatica*. (After Butler and Buckley.)

have discarded this family and redistributed the included genera. Thus we find *Urophlyctis* placed in the Cladochytridiaceae. Jones and Dreschler(68) and Bartlett(12) among others have called attention to the close affinity which exists between *Cladochytrium* and *Urophlyctis*.

Bartlett(11) includes in the Cladochytridiaceae four genera *Cladochytrium*, *Physoderma*, *Urophlyctis* and *Nowakowskiella*.

Fischer (43) grouped all the then known species in the genus *Cladochytrium*, and subdivided it into three sections, *Cladosporangium*, *Urophlyctis* and *Physoderma*. Cavers (21) among others has pointed out that the genus *Diplophysa* is equivalent to that part of the genus *Olpidiopsis* in which sexual reproduction has not been observed. Such a genus is obviously artificial, and those species which are therein included should be distributed among the genera *Olpidium* and *Olpidiopsis*.

The genus *Macrochytrium* described by von Minden (94) in 1916 suggests the final stage of the Cladochytridiaceae. *Macrochytrium botrydioides* grows on rotten fruit. The vegetative soma is relatively large and is attached by extensive rhizoids. It bears a single terminal branch from the ends of which a large spherical zoosporangium is formed. When mature, a lid is developed at the top through which the zoospores, still enclosed together in a delicate membrane, escape. Later this membrane ruptures and the contents are liberated. The zoospores possess a single flagellum.

In the character of the rhizoids and in the globose sporangium this species may be compared with the genus *Rhizophidium*. In the method of dehiscence of the sporangium, it is somewhat similar to the genus *Cladochytrium*. At the same time, the sporangium in some respects may be compared with that of the Saprolegniales, especially the Blastocladiaceae.

Summarising the views expressed so far, we may say that from the primitive *Proteomyxa* we have two lines of evolution entering the Chytridiales, the one in which penetration has been effected at an early stage giving us the *Olpidium* series and the Synchytriaceae, the other in which infection has been slow and consisted in the gradual sinking down of the sporangium into the host tissue. Here, starting with *Harpochytrium*, we can trace a series through the Rhizidiaceae to the Cladochytridiaceae which in turn may be connected to the Saprolegniales through the genus *Macrochytrium*.

Such a conclusion may be graphically represented by using as pegs the few types with which we are familiar, but we must realise that in all probability none of them represents the true ancestral type of the more specialised form to which they are said to have given rise. Such graphical representation can only be looked upon as a means to clarity, which in the absence of any geological evidence must necessarily be very imperfect.

It is necessary to say something more about the family Hyphochytridiaceae, to which *Macrochytrium* is said to belong. The family is

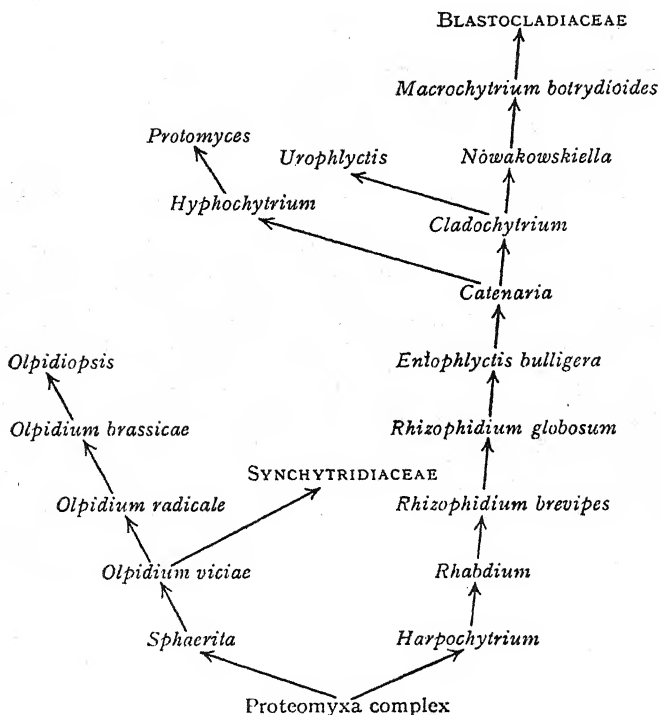


Diagram V

distinguished from the Cladochytridiaceae by the presence of a more definite mycelium and in the supposed differentiation into basal and apical regions. It seems likely however that such a distinction is rather a direct result of environment than one of phylogeny. *Macrochytrium* we may conclude developed such a basal structure in direct response to its habitat and the need for a strong rooting system to anchor a large zoosporangium. In *Hyphochytrium infestans* of Zopf (156) we find no such differentiation, in fact it consists of little more than a system of short mycelial strands swelling out here and there into zoosporangia.

The position of *Protomyces* has for a long time been one of considerable difficulty. The spores come to rest on the surface of the host, form a germ tube and give rise to a mycelium within the host tissue which is multinucleate and generally septate. After a while, certain segments swell up and receive the contents of that part of

the mycelium closely associated with them. Thick winter-resting sporangia are formed in this way. In the spring the contents divide up by vacuolation and cut out uninucleate portions. Nuclear division follows and each segment becomes converted into four spores. These are later liberated, and as far as is known, they are non-motile. It is very difficult to relate this genus to any other known form and its position must remain doubtful. It does however in many respects resemble the Hyphochytridiaceae and in particular *Hyphochytrium infestans*. In both the zoosporangia are formed by the swelling up of portions of the mycelium. In *Hyphochytrium infestans* however Zopf found that some of the zoospores possessed a single flagellum, though at the time of emergence from the zoosporangia these were apparently absent. The presence of septa in the mycelium is not unlike what has been described by Butler (19) in *Catenaria anguillulae*. In a general way it can be said that the genus *Protomyces* may be best regarded as related to this group, and probably originated somewhere near *Hyphochytrium*. Later, as a result of successful parasitism, it became secondarily modified.

Mention has already been made of the fact that certain of the Chytridiales have originated from the algae. A particular instance is afforded by *Rhodochytrium*. This genus was first described by Lagerheim (82) and later by Griggs (49), and in many ways may be related to the Protococcales through such a species as *Phyllobium dimorphum*. This species was described by Klebs (77) and is parasitic. It possesses chlorophyll, but has developed a complex rhizoidal system presumably in connection with its parasitic habit. It has been shown moreover that not only do the zoospores exhibit motion characteristic of the green algae, with two apical flagella, but that they also contain starch grains. Despite the attempt made by Griggs to connect the Synchytridiaceae with *Rhodochytrium*, on cytological grounds, and the fact that he considers the number of flagella is not a character of phylogenetic importance, it is difficult to see why the Synchytridiaceae should be derived direct from the Protococcales through *Phyllobium dimorphum* and *Rhodochytrium* rather than from the *Olpidium* series which they resemble equally well cytologically as well as in the flagellation of the zoospores. It seems preferable to consider *Rhodochytrium* to be a form derived direct from the algae sufficiently recently not to have lost some of its algal characters, such as starch and also the red pigment found in zoospores, and at the same time not of sufficient age to have given rise to any series of chytridiaceous types.

It is not necessary for the present consideration to make special mention of the many other genera which have been described and referred to the Chytridiaceae: many of them are only very incompletely known, and those whose life history is understood fall fairly well into line with the forms already mentioned. A few difficult types exist: such for example as *Polyphagus euglenae*. The life history of this organism has been carefully worked out by Dangeard (36) and by Wager (147). Some workers relate it to the Rhizidiaceae, connecting it with *Zygorhizidium Willei* or *Rhizoclomastium globosum* (111). It may be said that such forms show very little resemblance to any of the main lines of the Chytridiales, but stand apart as forms more comparable with the Proteomyxa or the Labyrinthulideae. We have not however sufficient evidence to compare them closely or to indicate any phylogenetic series.

#### DESCRIPTION OF PLATE VIII

Photomicrographs taken with Zeiss Seidentopf photographic eyepiece at tube length 115 mm. using Zeiss 2 mm. objective (Fig. 4); Zeiss 4.2 mm. objective (Figs. 1, 2, 3, 5, 6, 8, 9, 10); Beck 12.5 mm. (Figs. 11, 12) and Zeiss 16 mm. (Fig. 7).

- Fig. 1. *Rhizophidium* sp. attacking the cells of *Chlamydomonas*.  $\times 230$ .
- Fig. 2. *Olpidium brassicae*; zoosporangia showing exit tubes.  $\times 230$ .
- Fig. 3. *Olpidium radicale*; developing zoospores in host cells.  $\times 230$ .
- Fig. 4. *Olpidium radicale*; conjugation in host cells.  $\times 550$ .
- Fig. 5. *Physoderma zeae-maydis*; sporangia.  $\times 230$ .
- Fig. 6. *Protomyces pachydermus*; sporangia in host cells.  $\times 230$ .
- Fig. 7. *Urophlyctis alfalfae*; section through a nodule.  $\times 50$ .
- Fig. 8. *Polysiphonia fastigiata*; showing the haustoria entering the tissues of *Ascophyllum nodosum*.  $\times 230$ .
- Fig. 9. *Palaeomyces Horneae*; a fossil species from the Devonian strata.  $\times 230$ .
- Fig. 10. A fossil fungus with the spores of another species lying within.  $\times 230$ .
- Fig. 11. *Palaeomyces Gordonii*, v. major; sporangium.  $\times 75$ .
- Fig. 12. *Palaeomyces Gordonii*, v. major; sporangium.  $\times 75$ .

(To be continued)





Fig. 1

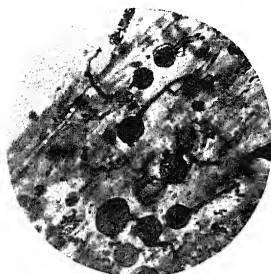


Fig. 2

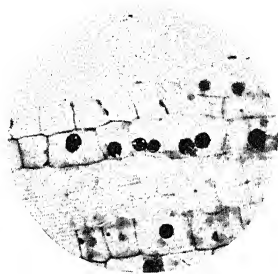


Fig. 3

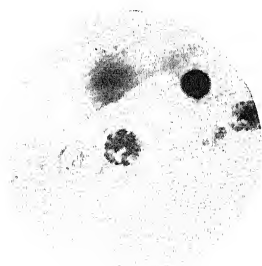


Fig. 4

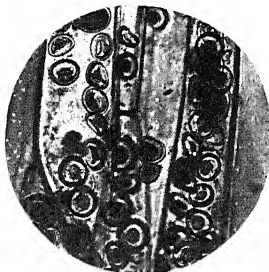


Fig. 5

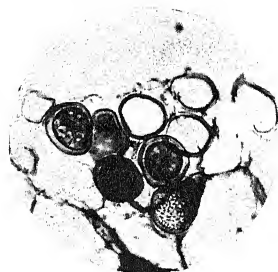


Fig. 6

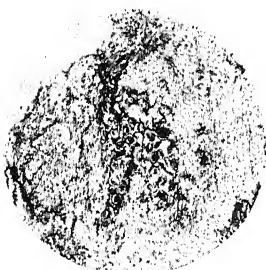


Fig. 7

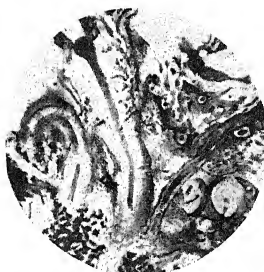


Fig. 8

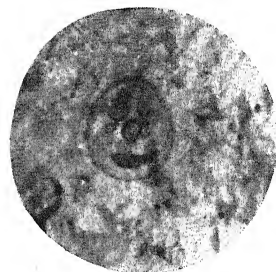


Fig. 9

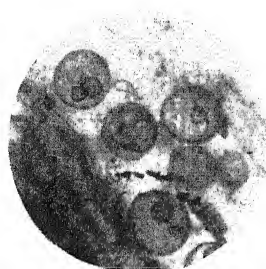


Fig. 10



Fig. 11

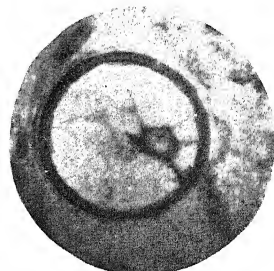
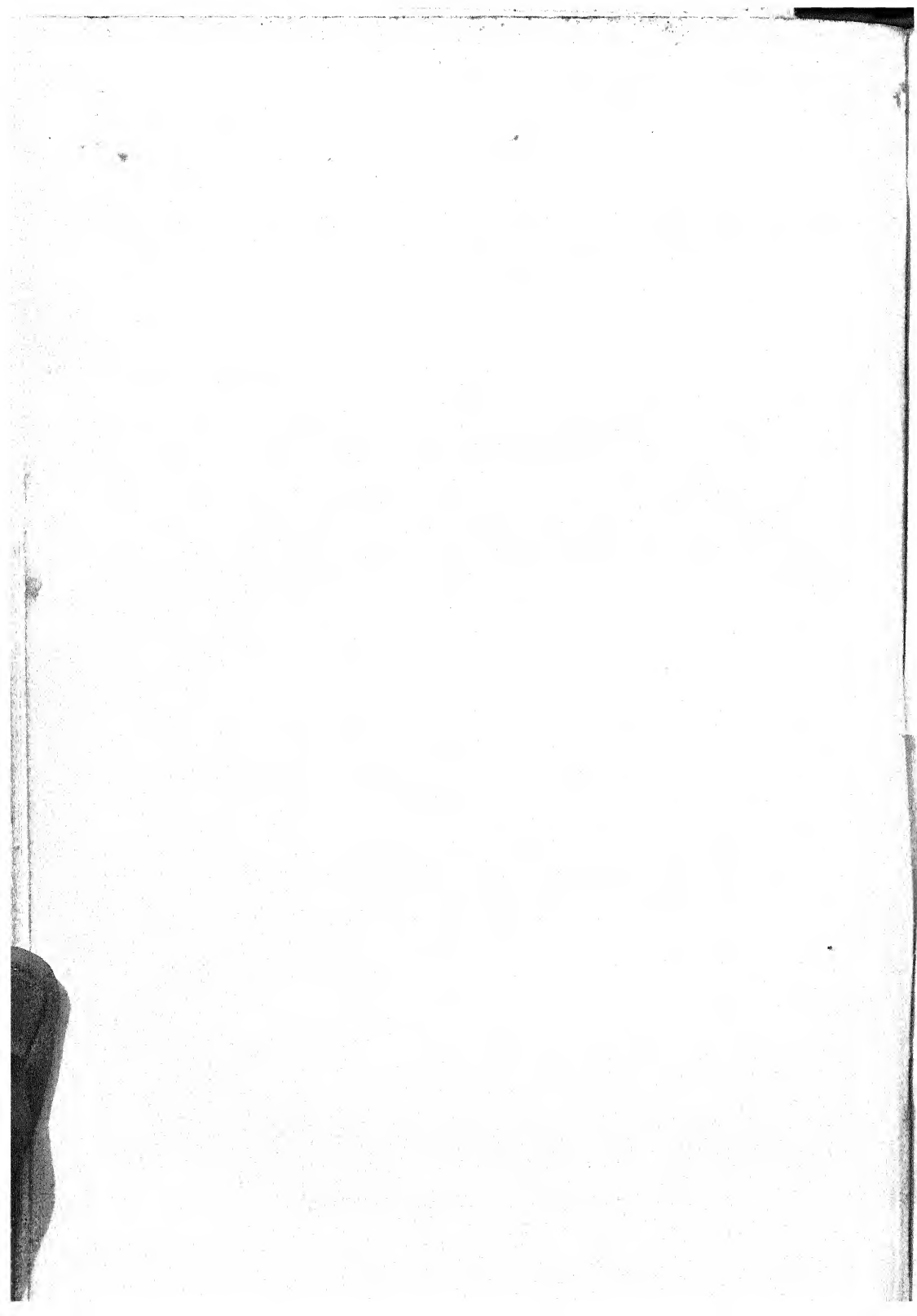


Fig. 12



# THE NEW PHYTOLOGIST

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## THE CAPTURE OF PREY BY THE BLADDERWORT<sup>1</sup>

A REVIEW OF THE PHYSIOLOGY OF THE BLADDERS

By ALEXANDER F. SKUTCH

(With Plate IX and 2 figures in the text.)

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### I. INTRODUCTION

FOR more than fifty years which have elapsed since the publication of the classic researches by Darwin and Cohn, *Utricularia* has been included among the insectivorous or, more properly, carnivorous plants. However, it was not until quite recently that anything positive has been known about the mechanism by which the bladders of this plant effect the capture of their prey—the original explanation of Darwin and Cohn was hardly satisfactory. The first announcements of the discovery of the active engulfment of the victims by the springing of a set snare appeared in publications not claiming the attention of botanists generally, and so for some time remained unnoticed, even by those actively engaged in the study of the bladders. The best evidence of the truth of this statement lies in the fact that the active sucking-in of the quarry has been *discovered independently no less than four times*, by observers in widely separated parts of the

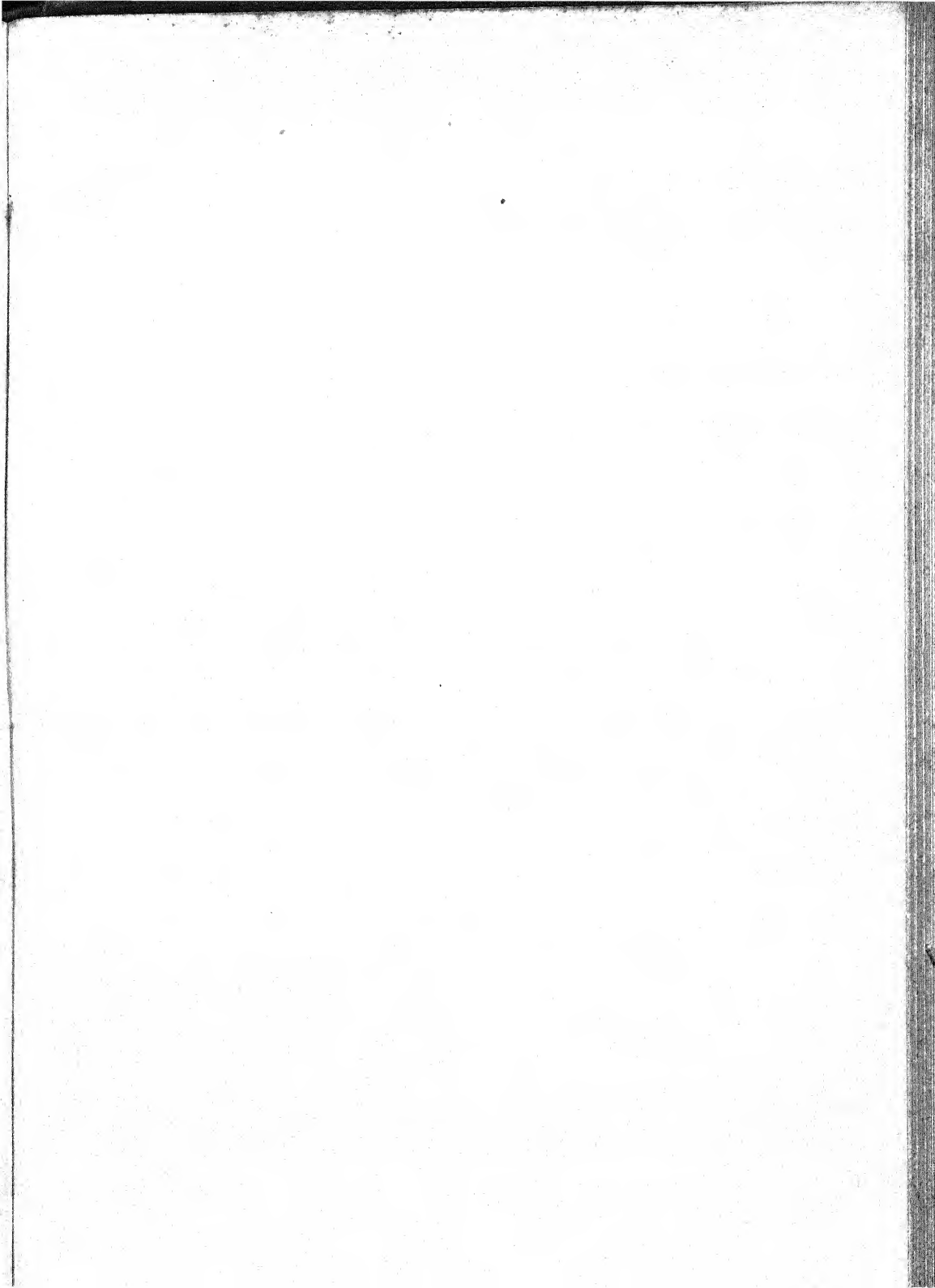
<sup>1</sup> Botanical Contribution from the Johns Hopkins University, No. 93.

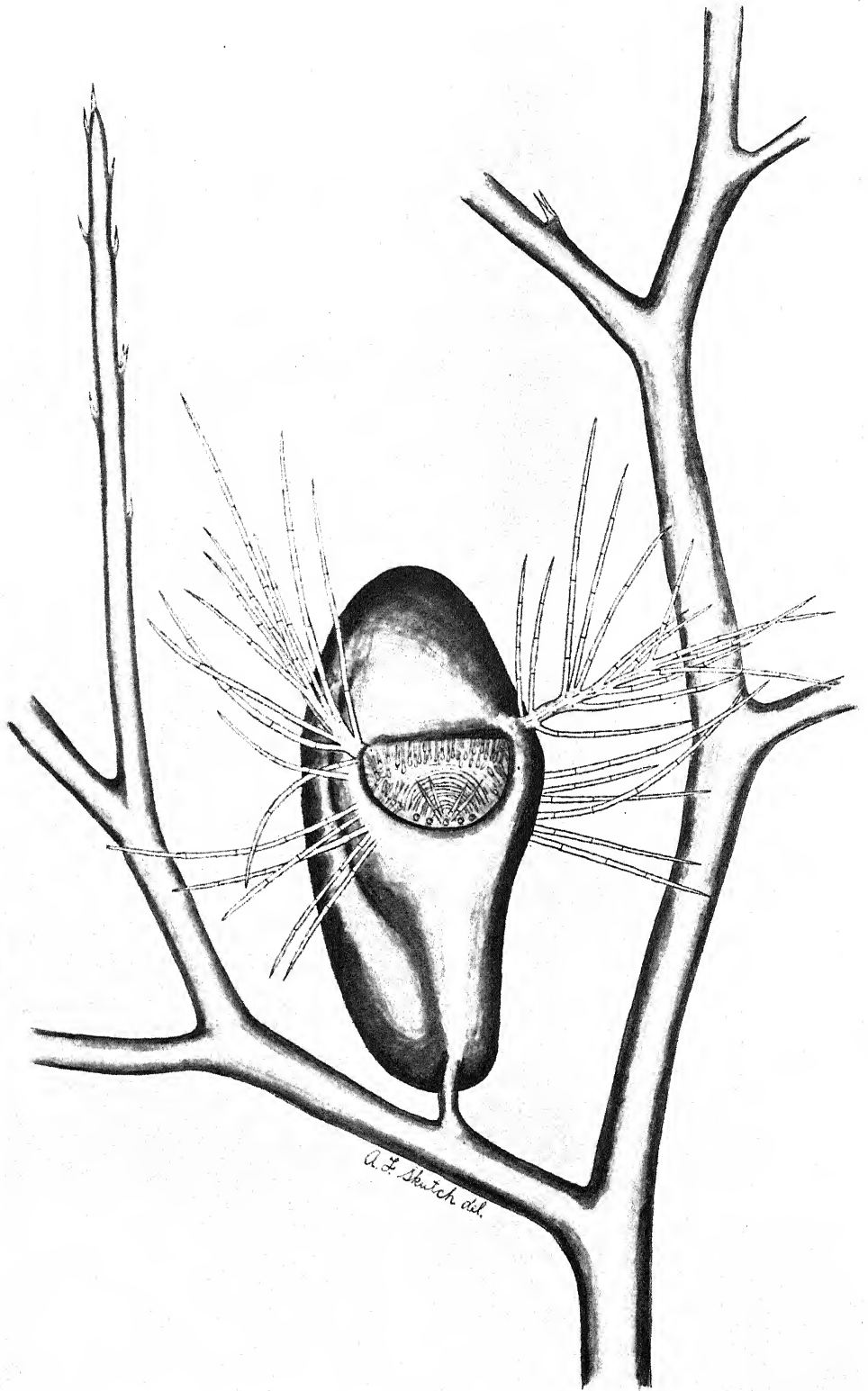
world. In view of the very general biological interest of *Utricularia*, which appears to be one of the most peculiar and highly specialised of insectivorous plants, and of the scattered nature of much of the original literature, it seems to be of value to bring together into a single article and to make accessible the most important results of previous investigations. This interest appears to be broadening from the application of the bladders to several new uses—as an unique object adapted to the study of the nature of the external membranes of submerged aquatics, a research which the recent discovery of the significance of hydropotes promises to make of considerable importance; and as a possible agent for the prophylactic treatment of waters infested by the larvae of malarial mosquitoes.

The writer was led to the investigation of this problem at the suggestion of Professor R. W. Hegner who, while studying the bladders in another connection, had discovered their sudden increase in volume upon touching the valve, and recognised the need of research on the physiology of this reaction. At the same time that the subject was tackled in the laboratory, a thorough search was made of the literature, which revealed that the necessity of investigating this behaviour of the bladders was perhaps not so urgent as the promulgation in a form more readily accessible of the results of investigations already made on the subject. The present paper is, then, the outgrowth of plans for experiments which for this reason never materialised. The writer has, however, satisfied himself by personal observation of the correctness of the salient points of the story as retold here. He desires to acknowledge his great indebtedness to Professor Hegner and to Professor B. E. Livingston for many helpful criticisms and suggestions.

## II. THE STRUCTURE OF THE BLADDER

The most complete accounts of the structure of the bladders of floating species of *Utricularia* are those of Cohn (5) and Meierhofer (27) for *U. vulgaris*, of Darwin (11) for *U. neglecta*, and of Goebel (17, 18) for *U. flexuosa*. Many other species have been more or less thoroughly studied and compared with these forms (see in particular Meierhofer, von Luetzelburg and Goebel). Withycombe (35) and Czaja (7) have given special attention to the structure of the valve and collar. The ontogeny of the bladders of four species has been carefully followed by Meierhofer, and Darwin, Kamienski (23) and Goebel have figured stages of their development. Perhaps the most detailed account of the structure of the bladder of a floating species is that by Meierhofer





A bladder of *Utricularia vulgaris* viewed from the front, showing the valve and the appendages surrounding the orifice.

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of *U. vulgaris*, and this is certainly the most completely illustrated of all.

The bladder of *U. vulgaris* is roughly lenticular in shape, and may reach 2.5 or 3 mm. in length. It is attached by a short, slender stalk, inserted on its ventral or adaxial surface, to a spot near the inner angle of a fork of the leaf, which is dissected in a filiform manner. A single leaf may bear a dozen or more bladders, but often carries fewer. Occasionally, at the height of summer, a hundred bladders may be seen on one large leaf, and Glück(16) gives as a maximum 209. The bladder, which is the morphological equivalent of a segment of a leaf, or at times of a whole leaf (Kamienski(23)), is bilateral in symmetry (see Fig. 1 and Plate IX). The dorsal line is elongated and strongly arched, the ventral shorter and almost straight. At the anterior end is found the roughly semicircular aperture, which lies on the ventral surface. This aperture is closed by a valve, the free, ventral margin of which lies against a thickened pad of cells, the "collar" of Darwin. From the sides of the aperture, in continuation of the dorsal surface, spring two long, slender, branched, multicellular appendages, which Darwin termed the "antennae," from their resemblance to the antennae of an entomostracan crustacean. Below the antennae, there is on either side of the aperture a row of several long bristles, a single cell in thickness and several in length.

The walls of the bladder are everywhere two cells in thickness, except for the collar and the vascular bundle. The latter branches as it enters from the stalk, sending a posterior division along the dorsal wall and an anterior along the ventral wall. These vascular strands lie in the median plane of the bladder, and extend nearly to the aperture. In a truly median, vertical section of the bladder, the walls would therefore appear several cells thick. The ventral branch of the bundle gives off short, blind branches to the right and left as it enters the collar.

The roughly semicircular valve is attached dorsally by the arc, except for a short distance on either side where the arc joins the straight edge, which also is free. It consists of two regions, a central semicircular portion which is convex outward in both horizontal and vertical sections, and a ring-shaped outer portion which is flat. The outer of the two layers of the wall consists of a restricted area of small cells situated at the base of the four bristles, near the middle of the free margin, and around this of larger cells with angular, zig-zag anticlinal walls. The inner layer is of larger and less compactly arranged cells, their long axes radiating from a region of small cells

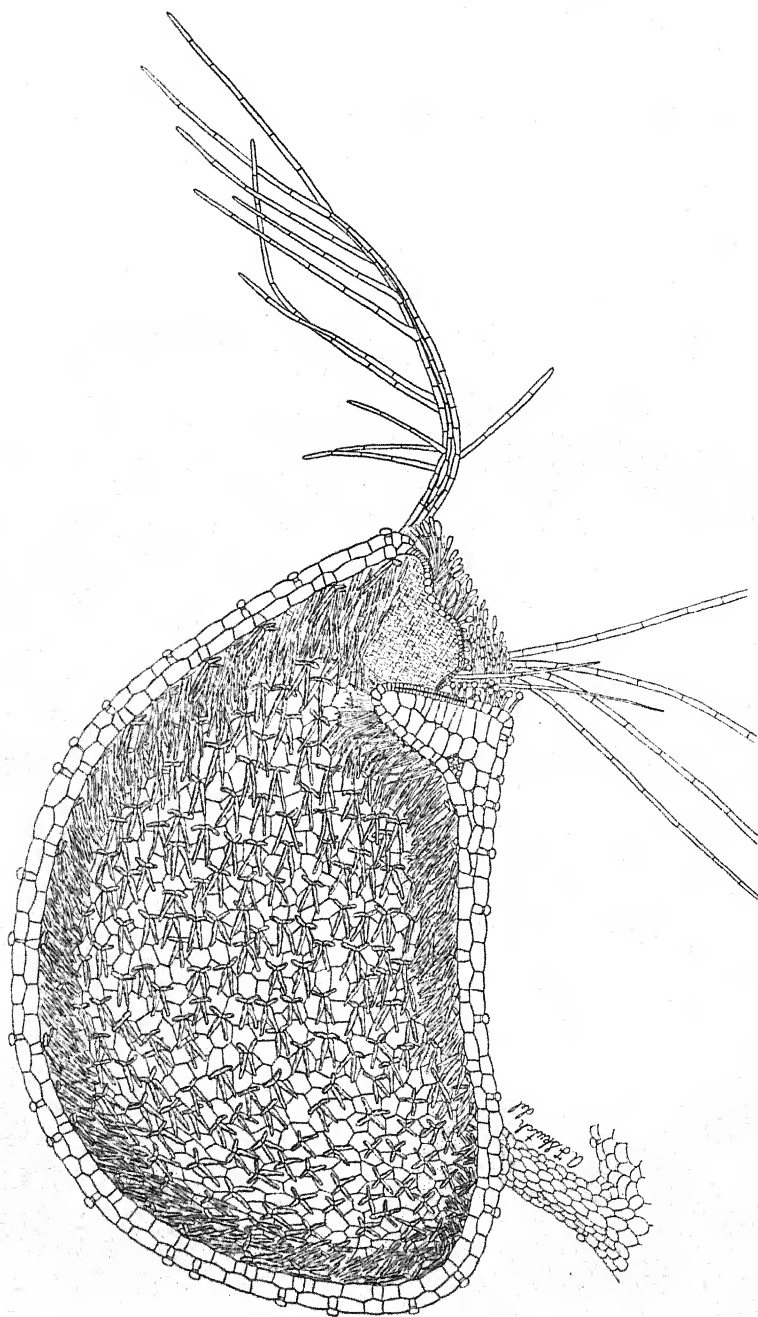


Fig. 1. A bladder of *Utricularia vulgaris* with one side removed to show the internal structure.



at the base of the bristles. Most of the cells of both layers have walls thickened by anticlinal ridges, which are more pronounced in the outer stratum, and the walls of the inner layer facing the cavity of the bladder are strengthened by broad thickenings which are continuous from cell to cell, and form conspicuous rings concentric about the bases of the bristles.

All parts of the bladder are studded with numerous trichomes, which are of great importance in the mechanism of the organ. These hairs may all be considered as modifications of a primary morphological type consisting of three cells, a stalk cell inserted in the wall, a short neck cell, and a head cell which may be variously modified. The whole trichome arises as a papillate outgrowth from a single superficial cell of the wall. The external walls of the bladder bear, in common with the leaves and the stem, glands in which the roughly spherical head is divided into two cells by an anticlinal wall. The greater portion of the inner wall is supplied with trichomes in which the head is divided into four cells, the "quadrifids" of Darwin. The arms, which are generally long and gently tapering, are grouped in pairs. Those of the longer pair point backward, away from the aperture, the shorter more or less sideways. The arms of all the quadrifids are longest near the aperture, and shortest near the stalk. On the inner side of the collar are found trichomes terminating in two long, tapering arms, which diverge from each other only slightly. The outer surface of the valve is richly supplied with hairs. Near its upper, attached margin are found the club-shaped hairs, with a very long stalk cell, short neck, and enlarged, cylindrical or clavate head. Proceeding toward the ventral margin the stalk cells become shorter, and the heads more spherical. Near this margin, the very large spherical or transversely elongated heads are practically sessile. In addition to trichomes of this type, the valve bears near its lower margin, and symmetrically placed on either side of the median line, two pairs of long, slender bristles, composed each of several uniseriate cells.

The collar, or sill, is composed of a cushion of large, parenchymatous cells, surmounted by a triple layer of specialised cells, which form a glandular epithelium. In a longitudinal section of the bladder, the epithelium appears to be made up of an upper and a lower layer of palisade-like cells, separated by a middle layer of flattened cells. Adjacent rows of cells are not fused by a middle lamella, for the pad is formed merely of closely packed trichomes of the typical structure. In surface view, the glandular heads are elongated parallel to the

length of the collar, so that they present their broader walls to the edge of the valve.

From the above description, the trap-like nature of the bladder should be at once evident. The valve is so contrived that it is free to move inward, but pressure against its inner wall only pushes it more firmly against the resisting collar, and escape from the interior of the bladder is impossible. Various functions have been ascribed by different authors to the several types of trichomes. The appendages surrounding the orifice, consisting of an antenna and the several bristles situated on each side and converging toward their insertion, were supposed by Darwin to act in the manner of a funnel, conducting to the aperture any animal which may happen to swim in among them. On the other hand, Hegner<sup>(21)</sup> states that they "seemed rather to hinder than to guide paramoecia to the opening, since many specimens that might have entered gave the avoiding action and swam away when they encountered these bristles." They have also been supposed to facilitate the access to the valve of creeping animals. The glandular hairs situated on the valve and collar secrete a mucilage which probably serves to attract small aquatic animals. The four-armed trichomes on the inner wall have always been regarded as absorptive in function, taking up the nutritive substances derived from the disintegration of the prey, and they also remove water from the lumen in "setting" the bladder. The external, two-celled glands are perhaps hydropotes, at any rate, they readily absorb various dye-stuffs (Czaja<sup>(7)</sup>).

### III. HISTORICAL SUMMARY

During the vegetative season, the rootless shoots of the free-swimming, aquatic species of *Utricularia* float just beneath the surface of the pond or ditch in which they are growing. The conspicuous flowers are displayed in the air at the summits of long peduncles. Earlier botanists thought that the small bladders borne on the finely dissected leaves served merely to impart to the plant the buoyancy requisite to remain afloat and to sustain the weight of the aerial inflorescence. According to their observations, these bladders usually contained air. At the end of the vegetative season, when the compact turion or winter-bud was formed from the apical growing point, the bladders became filled with slime or water, or else dropped off, allowing the plant to sink to the bottom. In the following spring, air-containing bladders appeared on the newly expanding shoot, and lifted the plant to the surface once more (A. P. DeCandolle, quoted by Goebel<sup>(18)</sup>).

To those of this opinion the function of the bladders was the same as that of the peculiar flotation-apparatus at the base of the inflorescence of such highly specialised forms as *U. stellaris* and *U. inflata*. An expression of this view may be found in the earlier editions of Asa Gray's *Structural and Systematic Botany* (20), p. 445). Even in 1879, after the appearance of the researches of Darwin and Cohn, Drude(12), while admitting that the bladders serve as pitfalls for animals, was inclined to regard their supposed function as a flotation apparatus as of equal importance, and consequently to view their ecological significance as two-fold.

However, if all of the bladders are removed from a bladderwort plant it will still float, as do many other aquatic plants without bladders; thanks to the air contained in the intercellular spaces of the stem and leaves, every portion of the plant is of itself buoyant. Moreover, one sometimes finds in the autumn plants which have shed all their bladders, but have not thereby lost their buoyancy (Brocher (1)), and in the spring turions sometimes rise to the surface before the young leaves have developed bladders (Goebel(18)). In some species, as *U. intermedia*, the bladders are not uniformly distributed over the plant, but are borne only on specialised leafless branches which are positively geotropic (von Luetzelburg(26)), and grow vertically downward beneath the surface of the water. Later students have shown that the *Utricularia* bladders do not usually contain gas-bubbles in their lumina, and hence cannot greatly decrease the specific gravity of the plant as a whole. Nor does the old explanation account for the presence of the bladders of the terrestrial bladderworts, which constitute the majority of the 250 or more species making up this genus, all except one of which possess bladders, albeit usually not in such abundance as in the case of the aquatic species. The single recorded species which is devoid of bladders is *U. neottioides*, which grows attached to rocks in the rapids of streams in Paraguay and Brazil (von Luetzelburg(26)).

According to Darwin, the Crouan brothers(6), pharmacists and amateur botanists of Brest, were the first to record, in 1858, the presence of small aquatic animals within the bladders of *Utricularia*. Ten years later, Holland(22), according to Darwin, observed the same thing on English plants. In 1875, Darwin(11) published an account of his experiments made principally on *U. neglecta*, and showed that the bladders of this plant often contain small crustacea, insect larvae, etc., which die in the prison, and he concluded that the dead bodies of these supply food to the plant. As it has turned out

through later work, he rightly regarded *Utricularia* as a genuine carnivorous plant. In the same year, Mrs Treat<sup>(83)</sup> observed mosquito larvae and crustacea within the bladders of an American species, and followed the actual entrance of a larva into a bladder. Cohn<sup>(5)</sup> was stimulated by reports of Darwin's work on insectivorous plants to undertake the investigation of *U. vulgaris*, and satisfied himself that he was dealing with a carnivorous species. He fortified his deductions from structure and from analogy with other rootless plants by experiments and observations on living and herbarium material. Since the publication of the work of these pioneers, observations on the bladders of many species of *Utricularia* have multiplied to such an extent that there can be no doubt of their carnivorous habit.

The next step was to discover the method by which the bladders engulf their prey, and in this endeavour all of the earlier observers were equally unsuccessful. It is of much interest to follow Darwin's account of his efforts to clear up the problem. "As I felt much difficulty in understanding how such minute and weak animals, as are often captured, could force their way into the bladders, I tried many experiments to ascertain how this was effected. The free margin of the valve bends so easily that no resistance is felt when a needle or thin bristle is inserted. A thin human hair, fixed to a handle, and cut off so as to project barely  $\frac{1}{4}$  in., entered with some difficulty; a longer piece yielded instead of entering. On three occasions minute particles of blue glass (so as to be easily distinguished) were placed on valves whilst under water; and on trying gently to move them with a needle, *they disappeared so suddenly that, not seeing what had happened, I thought that I had flitted them off*; but on examining the bladders, they were found safely enclosed. The same thing occurred to my son, who placed little cubes of green boxwood (about  $\frac{1}{60}$  in., .423 mm.) on some valves; *and thrice in the act of placing them on, or whilst gently moving them to another spot, the valve suddenly opened and they were engulfed*. He then placed similar bits of wood on other valves, and moved them about for some time, but they did not enter" (<sup>(11)</sup>, p. 405. The italics are my own; how nearly Darwin came to the true explanation will appear in the sequel). He also mentions the eventual disappearance of particles of blue glass, lead shavings, etc., which had rested for some time on the valve without being swallowed. In one case a cube of green boxwood remained on the surface of a valve for 19.5 hours without entering, but was found inside 3 hours later.

Darwin already knew that *Dionaea*, *Drosera*, and *Pinguicula* execute active movements either in the capture or in the digestion of their prey, and acting upon the suggestion offered by these, he tried to discover whether the valve of *Utricularia* is irritable. He scratched the surface with a needle, or rubbed it with a fine camel-hair brush, but was unable to observe that it responded by opening. He observed the same lack of response when he maintained the bladders at unusually high temperatures (26–54° C.), expecting thereby to increase their irritability. He concluded "that animals enter merely by forcing their way through the slit-like orifice; their heads serving as a wedge." He supposed that minute animals habitually seek to intrude themselves into small cavities, in search of food or protection, but expresses his surprise that such small and weak creatures could push the valves inward.

Cohn<sup>(5)</sup> had already concluded, as a result of his own observations, that the *Utricularia* bladder is not endowed with irritability, and that the captured animals force their way into it, which he conceived they must do against an internal pressure which holds the valve against the collar and prevents their subsequent escape. The explanation of Büsgen<sup>(4)</sup> differed from this in detail only. In the natural orientation of the bladder the external surface of the valve often faces upward. Büsgen believed that the animal did not endeavour to force its way inward, as Darwin had supposed, but that the weight of the creature, as it crawled over the valve, bent it downward and inward, just as a small pebble would do if pushed about on its surface. He compared the deformation of the valve, and its elastic recoil after being pushed inward, with the behaviour of a rectangular piece of flexible cardboard, bent into a semi-cylinder and supported at the sides. A slight pressure exerted upon the convex surface at either end creates a deep furrow, which straightens out immediately upon the release of the force.

And so the matter rested for the third of a century, until it was taken up again in 1911 by the entomologist Brocher, and this time with a more fruitful outcome. Brocher<sup>(1)</sup> was impressed with the fact that none of the previous investigators had observed in detail the actual course of events as an animal entered a bladder, and undertook to witness this occurrence for himself. He began by placing a copepod, injured slightly so that it could not slip away, upon the valve of the bladder. In most cases, nothing came of this procedure, but several times, as he manoeuvred the animal upon the valve with a needle, it suddenly vanished, just as Darwin's particles of blue

glass had vanished, and was later found inside the bladder. Next he tried projecting a small crustacean against the valve from a pipette. Usually this endeavour was also fruitless, but upon one occasion a cladoceran, which had mounted to the top of the water column in the pipette, was engulfed by the bladder, along with the bubble of air which followed it from the tube, and to the surface of which it adhered. The entry of the air bubble indicated to Brocher that *the bladder experiences an instantaneous increase in volume at the time of ingesting its prey*. In other words, it sucked in the animal and the bubble as the expanding rubber bulb sucks water into a pipette. He further observed that the swallowing of the prey occurred only when contact had been made with the region of the valve at the base of the four long bristles, and that it never succeeded in a bladder which contained a bubble of gas, or had already captured an animal. Careful examination convinced him that the functional bladders were slightly concave on the sides, while those which would not react were somewhat convex in the same region. The change from the concave to the convex condition occurred at the time when an animal was captured, and was responsible for the change in volume. The difference between the two states is clearly indicated in his camera-lucida outlines (see also photographs by Merl(28), Figs. 1 and 2, and Fig. 2 in this text). Moreover, Brocher could sometimes observe that the bladder trembled slightly, as though it had been jarred, at the time it enlarged to suck in its prey.

Brocher discovered the reason for his numerous failures, during the earlier part of his investigation, to observe the capture of prey. Examination of bladders which had never been removed from the water showed that almost without exception they were free from gas-bubbles. However, if a plant is merely lifted from the surface, and immediately returned to the water, a large proportion of its bladders will be found to contain air-bubbles. The bladder expands as it passes through the surface film and, since the expansion is completed in the air, a bubble of gas is sucked in. Often he could discern a faint clicking sound as a bladder expanded. The plants with which he had previously experimented had been transferred through the air, and many of them had thereby exhausted their power of expanding and had become inactive. By using the proper precautions and transferring the plants from the pond in which they grew to the experimental vessel without bringing them out of water, a larger number of bladders were secured in the active condition. Upon stimulating such bladders at the base of the four bristles of

the valve, it was possible to obtain the expansion in almost every case.

Brocher demonstrated, then, that the bladder is not passive in the capture of its prey, the animal forcing its way inward past the yielding valve, but on the contrary, upon receiving the proper stimulation, it undergoes an increase in volume, which causes an inflow of water, carrying with it any small animal so unfortunate as to have released this chain of events. Brocher's paper was published, however, in a journal which did not claim the attention of many botanists, and in consequence escaped general notice.

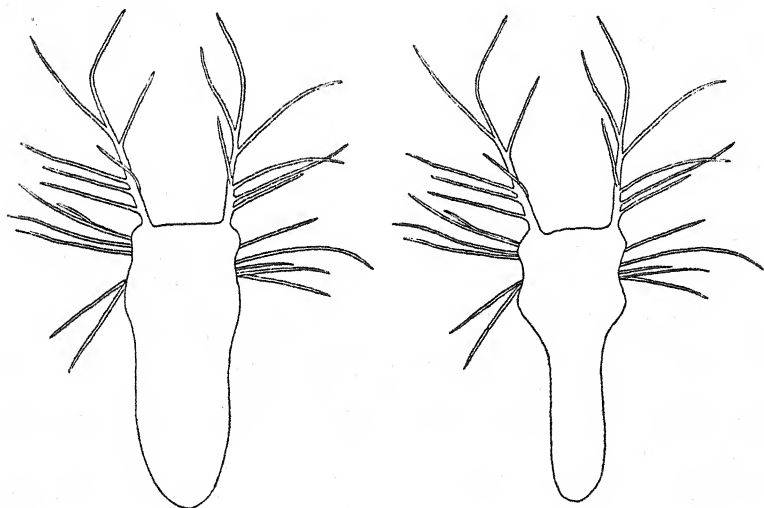


Fig. 2. The same bladder of *Utricularia vulgaris* before (right) and after (left) touching the valve with a needle. Camera-lucida sketches.

While distributing for class study specimens of an undetermined Indian species of *Utricularia*<sup>1</sup> with very large bladders, Ekambaram (13) noticed "light crackling sounds resembling the ticks of a watch" which emanated from the plants as he lifted them from the vessel in which they were growing. A series of observations satisfied him that the particular organs of the plant responsible for the noise were the bladders. By a comparison of the condition of the bladders before and after their removal from the water, Ekambaram discovered that a change of configuration had taken place, and concluded that the almost explosive alteration of form was accompanied by the

<sup>1</sup> Ekambaram states that this species was "very near *U. flexuosa*."

sounds which had originally attracted his attention. The unmolested bladders were usually "nearly empty, with walls closely adpressed against each other, so that there was very little cavity inside, and the bladder as a whole was biconcave." Many of those which had been lifted from the water were greatly distended, had convex lateral walls, and contained a bubble of air. The change from a less to a more spherical shape was accompanied by a considerable increase in volume, and this sudden distension could be induced by touching with a needle one of the bristles inserted on the valve. Ekambaram concluded that the bladders are irritable, and capture their prey by actively enlarging their volume and sucking it in, which they do when the sensitive bristles are stimulated by the contact of a small aquatic animal.

The late Dr C. L. Withycombe<sup>(34)</sup> made the third independent discovery of this phenomenon. In 1916, as a youth of eighteen, he published an account of his observation of the manner in which the bladder sucks in its prey, which he had made with the aid of only a hand-lens. In 1925, Hegner<sup>(21)</sup>, while studying the fate of unicellular organisms entrapped by the bladders, discovered the same reaction for the fourth time, entirely without knowledge of the work of the previous observers. From a historical standpoint, it is remarkable that this phenomenon, which uniformly escaped the keen observation of the many early students of the bladders, should have been discovered on four distinct occasions, by naturalists of four different nationalities each unacquainted with the others' work, and all within the period of fifteen years. Of the earlier students, Darwin came the closest to observing what actually occurs. He had remarked that different bladders vary greatly in thickness and in the amount of water they contain, but failed to realise the great significance of this circumstance. However, had he been a little more diligent in following up the unexplained disappearance of his glass and boxwood particles, he must undoubtedly have been led to the true solution of the problem.

#### IV. THE MECHANISM OF THE BLADDER

The discovery by Brocher of the actual phenomenon attending the capture of prey by the bladder represented a great advance towards the solution of "le problème de l'Utriculaire," and for the second time made this plant a special object of investigation. The next step was to study in more detail the conditions under which the dilation takes place, and to discover the forces responsible for this



sudden change in configuration—to determine its mechanism. Is the sudden expansion of the walls the response of irritable tissue to a stimulus, or is it merely the result of the mechanical release of certain pre-existing strains? If the former alternative is correct, what is the nature of the reaction, and which are the motor cells? If the latter, which are the strained tissues, and how are the strains engendered? In the following discussion, we shall for brevity refer to Brocher's phenomenon as "the bladder reaction," or more briefly "the reaction," without meaning by this term to imply either that it is a manifestation of irritability, or that it is a purely mechanical change.

Already Brocher had framed an explanation of the process; he assumed that during the growth of the bladder the walls increase in area without a corresponding increase in the liquid content of the cavity. As a consequence, the bladder departs from the spherical shape, which represents the smallest ratio of surface to volume in a solid object, and the lateral walls become concave. They are strained in this position, and by virtue of their elasticity tend to spring outward, which they do if the bladder is punctured by a needle, allowing the influx of water through the perforation. But since the valve fits tightly against the collar, and the bladder is perfectly sealed, the contents of the cavity cannot normally be augmented by water from without, and therefore remain under negative pressure in respect to the medium. However, when the valve is stimulated by the contact of a solid object with the region at the base of the four bristles, it contracts, pulling away from the collar, and producing an opening which allows the influx of water. The strained lateral walls are now free to spring apart, and the inflowing current carries along with it the animal which has sprung the trap by impinging against the valve. Occasionally the valve closes before the walls of the bladder have reached their position of equilibrium, in which case a second reaction is possible. Brocher never observed the recovery of the "set" condition by a fully expanded bladder, but suggested that in a state of nature it might take place after several hours.

In Brocher's interpretation of the reaction of the bladder, its release from the set position is conditioned by the special irritability of the valve. He assumed the existence of a motor tissue not, it is true, to account for the springing asunder of the walls, which results from their elasticity, but for the opening of the valve which must precede this expansion. Ekambaram<sup>(13)</sup> also believed the valve to be irritable, but thought the perceptive faculty to be localised

especially in the bristles (six in the species he worked with) rather than in the region at their base. Before definitely accepting either view, it was necessary to investigate more closely the conditions under which the reaction proceeds, and to determine if the bladder behaves in a manner similar to other objects known to be irritable, such as the leaves of *Mimosa*, *Aldrovanda* and *Dionaea*, the stamens of *Centaurea* and *Berberis*, etc. Many experiments in this connection have been carried out by Merl.

Merl (28) used for his investigations bladders of *U. vulgaris* and *U. flexuosa*, severed from the leaves and floated, ventral side up, in a watch glass where they could be observed by means of a binocular or a hand lens. He determined that such separated bladders react just as well as attached ones. The reaction may be secured by touching with a needle *either the bristles or the valve itself in the region of their insertion*. Young, immature bladders do not respond. The increase in the volume of the bladder attending the reaction may be demonstrated by touching the hairs with a capillary pipette containing mercury or a coloured solution; a drop emitted from the pipette at the proper time is sucked in, even if the end of the pipette does not pass within the aperture. Isolated bladders could be observed to give a short jerk forward through the water as they expanded, the physical reaction to the influx of water. The whole reaction usually takes place in a flash and it is impossible to observe the movement in detail, but when the rate of activity is particularly low it may require 1-2 seconds for its completion, and its components may then be followed. The four bristles on the valve approach until their apices are in contact, at the same time moving backward. The edge of the valve moves inward until a semilunar aperture between it and the collar is visible, then immediately snaps forward. Meanwhile the lateral walls have dilated. The strength of the reaction is conditioned by the intensity of the touch, and if very slight contact is made with the bristles, the walls do not always completely expand. Upon a second, stronger stimulation such bladders will dilate further, as Brocher already had observed. The fullest possible dilation of the walls does not follow the ordinary gentle contact. If, however, the valve is forcibly pushed inward, or if the wall is punctured, the bladder becomes extremely swollen in appearance, and the walls assume a much greater convexity than they do as a result of simple contact with the bristles (see Merl, Figs. 1 and 2).

Merl showed that after reacting a bladder could again recover its "set" condition, and become ready for a second discharge. He

determined the period of recovery to be 15 minutes, but a longer rest period allows a greater concavity of the lateral walls to be attained. Withycombe<sup>(35)</sup> later found that the time necessary for complete recovery was related to the temperature, and that at the optimum temperature at least 30 minutes was required by the bladder of *U. vulgaris*. Czaja<sup>(9)</sup> agreed with Merl that 15 minutes is sufficient time for recovery, although he found in his earlier work<sup>(7)</sup> that full recovery required 30 minutes. Hegner<sup>(21)</sup> found 20 minutes the minimum period required for recovery. The rate of the process undoubtedly varies with the state of the material and the external conditions. Merl secured the reaction and recovery of a single bladder 14 times in 3 days. He proved by camera-lucida sketches that no change in size accompanies even repeated recovery, but the re-set bladder has the same dimensions as before reacting. Recovery takes place even if the bladder is abnormally enlarged by the forcible opening of the valve, but naturally requires a longer time under these conditions, since more water must be removed from the interior. Punctured bladders never become set again. The presence of nutritive substances does not inhibit the simultaneous recovery of the bladder, and a discharged bladder into which a piece of raw meat had been introduced was set again after 1.5 hours. Finally, it is not necessary for the bladders to be submerged, but they may react after being kept for a day in air in a moist chamber, and they remain capable of response even when the external pressure is reduced by the exhaust pump.

Ekambaram<sup>(13)</sup> showed that a bladder could be "set" by mechanical means. He pressed out most of the water by compressing the bladder between forceps, the water escaping through a slit which appeared between the valve and the collar. Upon releasing the pressure, the cavity became filled with air which entered from the intercellular spaces of the wall. After expelling this air several times by compression, until no more entered from the intercellular spaces, the bladder remained in a set condition, and responded normally to contact with the bristles. By this manipulation the same bladder could be several times set and released. This behaviour of the bladder favours the conclusion that variations in the turgor of the cells of the wall are not responsible for bringing about the set condition, and therefore are not involved in its release.

Hegner<sup>(21)</sup> made some determinations of the relative volumes of the compressed and the dilated bladders. He chose 15 large compressed bladders, and the same number in the distended condition.

After drying the exterior with filter paper, he withdrew the contents of each set by means of a fine capillary pipette. It was found that the volume of water removed from the distended bladders was 88 per cent. greater than that withdrawn from the compressed ones. In other words, the bladder upon expanding may draw in a volume of water equalling approximately 88 per cent. of that already contained in the lumen; and during the process of resetting about 47 per cent. of the water contained in the dilated bladder is somehow expelled. In attempting to gain some information as to the amount of suction produced by the bladder in expanding, Hegner found that a dead paramoecium placed 2 mm. from the orifice was drawn in when the bladder was caused to react. A dead insect larva 2 mm. long was completely engulfed when it was pushed against the valve, as was a living one of similar length when it touched the valve. Another observation which indicates the strength of the inrushing current was made by the writer. An imprisoned ostracod, which happened to be in the anterior end of the bladder, was jerked violently inward as the bladder expanded.

Merl was unable to cause the bladders to react by any kind of mechanical stimulation other than by contact with the bristles or the region of the valve at their base. Severing the supporting leaf from the plant, or the bladder from the leaf, brought about no response, and bladders just severed at the stalk were as strongly contracted as unmolested ones. By puncturing the wall with a needle the bladder was made to dilate very strongly, but apparently the valve did not open, the water entering merely through the perforation in the wall. Later Withycombe showed that a punctured bladder expands only after the needle has been removed, but does not alter its shape so long as the instrument plugs the hole and prevents the inflow of water through it. Attempts to cause the reaction by electrical stimulation were equally unsuccessful. None of the many chemicals applied to the bladder called forth response, even if they were used in strong enough concentration, or allowed to act for sufficient time, to cause injury. In all of these particulars the behaviour of *Utricularia* is strikingly at variance with that of most organs which respond to stimulation by a motor reaction. The leaf of *Aldrovanda vesiculosa* affords the best material for the comparison of an irritable organ with the bladder of *Utricularia*, since this is the only submerged plant known to give a rapid motor response, and is also insectivorous. Czaja (10) has recently investigated this reaction, and finds that the halves of the lamina close together as

a result of electrical and chemical, as well as mechanical, stimulation (see also Brown and Sharp<sup>(2)</sup> on *Dionaea*).

Having failed in his attempts to cause the bladders to react by means other than mechanical contact with a portion of the valve, Merl next endeavoured to demonstrate their sensitivity by showing that a state of rigor could be induced. He first tried to throw them into heat- or cold-rigor. He found that bladders of *U. vulgaris* still responded after being kept for 24 hours at 45° C., and after one hour at 48.5° C. These bladders were killed by exposure to 60° C. A few bladders of *U. flexuosa* still reacted after an hour at 50° C., while a temperature of 53° C. was fatal to them. Merl concluded that the maximum temperature permitting response was practically continuous with that supporting life. Attempts to produce rigor by cold met with the same negative results. A few bladders of *U. flexuosa* were in condition to react after 1 hour 20 minutes at 1° C., although many were injured by this temperature. Bladders of *U. vulgaris* reacted after being maintained at 0–2° C. for  $\frac{1}{4}$  hour. At the lower, as at the higher temperatures, the limit for reaction is apparently the same as for life. Ether, chloroform, mercuric chloride, alcohol, chloral hydrate and brucin were employed in attempts to produce rigor by chemical agents, but these substances prevented the response to mechanical stimulation only when they killed or severely injured the bladder. Several of these substances were found by Czaja to cause rigor of the leaves of *Aldrovanda*. The much more numerous quantitative experiments of Czaja<sup>(9)</sup>, which will be discussed in more detail in another connection, overwhelmingly support the same conclusion, although in a few instances conflicting evidence was obtained.

The experiments of Merl make untenable any theory of the reaction which postulates sensitivity (understood of course, in the meaning of the sensitivity of *Mimosa* or *Dionaea*) on the part of any portion of the bladder essentially involved in the process. Nevertheless, Withycombe, who states that his own experimental work supports that of Merl, persisted that the valve of the bladder includes a motor tissue which contracts on stimulation. If we admit this contention, *Utricularia* is unique in this respect, for every other adequately investigated motor tissue which reacts to contact responds also to other forms of stimulation, and may be thrown into a state of rigor by various means. Merl himself came to no definite conclusions as to the mechanism of the reaction, but suggests among other things that the bladders may dilate as the result of the

disturbance of a system in unstable equilibrium, which exists in the set condition. This is an idea which has been expanded and elaborated by Czaja.

Czaja (7) began by considering the elastic strains present in the walls and the valve. Cohn had already shown that if the peristome, including the collar, is removed, the bladder broadens laterally (Cohn (5), Fig. 8). If a bladder is cut in the median, vertical plane by an incision beginning at the anterior end and extending posteriorly as far as the stalk on the ventral side, and an equal distance on the dorsal, the two halves gape apart laterally. Thin cross-sections, made just behind the peristome, expand toward the sides, indicating a tendency of the lateral walls to become more convex. These strains are a result of the size and distribution of the cells of the wall. The mechanical structure of the valve is very carefully considered, but for details reference should be made to Czaja's figures and description. It must suffice here to state that as a result of its outward convexity, the more its sides are forced together by the incurved walls, the more tightly its free margin is pressed against the sill. Any inward displacement of the valve results in an elastic strain and a rapid recoil, which may be understood by referring again to Büsgen's bent cardboard model (p. 269).

It was also necessary to investigate the closure of the bladder by the valve. If the gradual approach of the lateral walls during the transition from the sprung to the set condition is brought about by changes in the shape of the wall cells caused by variations in their turgor, it should be independent of a difference in pressure between the external median and the lumen of the bladder, and should proceed whether or not the bladder is completely sealed. If the setting of the bladder is contingent on such pressure differences, an incompletely sealed bladder ought to remain permanently sprung. Czaja inserted a hair between the valve and the collar, and found that the bladder remained permanently in the dilated condition, and was incapable of reacting. After several days, the hair was removed, and the bladder became set and responded soon after, indicating that no injury had been incurred. The bladder, then, is normally hermetically sealed, and this may be demonstrated in another way by immersing it in a coloured solution (eosin, congo-red or methyl-blue), in which it may remain for days without the slightest penetration of the colouring matter into the cavity (see also Merl (28) and Withycombe (35)). However, Hegner's results are somewhat at variance with this conclusion: he found that eosin, carmine and indian ink penetrated the bladders

within  $2\frac{1}{2}$  hours. If a hair has been inserted beneath the valve, the dye penetrates rapidly. The very perfect seal is doubtless made possible by the rich secretion of mucilage by the glands of the collar.

Czaja next showed that the whole bladder is enclosed in a semi-permeable membrane. Prat(30) has since demonstrated that the entire plant is covered by such a membrane, and the researches of Riede(31) and others have pointed out the error of the once prevalent idea that the peripheral cells of submerged aquatics are everywhere in free communication with the medium. The osmotic pressure of the cells of the wall of a *Utricularia* bladder is equal to that of a  $M/5$ - $M/4$  solution of  $KNO_3$ , as may be determined by observations on the plasmolysis of halved bladders. However, glycerine or sucrose solutions of no concentration will produce normal plasmolysis of the cells of whole bladders which have been immersed in them. Placing the bladders in hypertonic solutions of these substances causes the walls to become more and more concave, until finally, with sufficient concentration, the opposite walls are brought into contact, and the lumen disappears. When this occurs a light disc is evident in the centre of a bladder viewed from the side by transmitted light, and a dark, median, vertical line may be seen from the front (see Czaja (7), Figs. 7 and 8). With concentrations of glycerine up to 30 per cent. the tension in the cavity increases, but with higher concentrations the bladder totally collapses, and not until this occurs do the cells of the wall become plasmolysed. The membrane surrounding the bladder prevents the access of the solute directly to the protoplasts; as the latter lose water through the external membrane in response to the existing concentration gradient, they make good their loss by absorbing the normal liquid from the cavity, and until this is exhausted plasmolysis cannot occur. When placed in a concentrated solution of a substance to which the membrane is permeable, the bladder becomes greatly distended, and appears to be dead. In such cases, the external membrane is completely destroyed, and it is impossible to maintain in the lumen the negative pressure necessary to hold the lateral walls in the "set" position. Merl showed that if a 5 per cent. solution of glycerine is introduced into a bladder the wall cells plasmolyse, but the excess osmotic pressure within causes the whole bladder to imbibe water from the medium, and it becomes greatly inflated. Not only does the valve completely shut off free communication between the external and internal fluids, but interchange through the walls is regulated by a selectively permeable membrane.

When the bladder is placed in a hypertonic solution, the inflection of the walls causes the valve to be wedged strongly against the collar. If the over-tension produced within the bladder is only slight, the valve may be forcibly opened with a needle, but if this is allowed to proceed too far, it is impossible to budge the valve, and efforts to do so result only in destroying it. The equilibrium between internal and external forces becomes more stable as the tension increases above the normal.

Finally, it may be demonstrated that the quadrifid hairs of the interior absorb substances from the cavity. Darwin long ago supposed that their function was that of absorption, and they have been found to take up dyes such as methylene blue with great readiness. That they are actually responsible for the removal of the large volume of water necessary to set the bladder still requires fuller experimental proof. The four bristles on the valve act as a lever mechanism. The displacement of one of them causes a reaction much more readily when the bristle is bent downward than when it is bent sideward to an equal degree. They may be regarded as structures adapted to effect a considerable deformation of the free margin of the valve by means of a slight applied force, an assumption to which their structure lends support (see Merl<sup>(28)</sup>, Fig. 3). The downward movement of the bristles breaks the contact between the valve and the collar. The antennae and the hairs along the sides of the peristome are not essential to the reaction, which proceeds normally when they are cut away.

We may now form a picture of the entire process and, starting with a bladder which has just expanded, trace its recovery and its subsequent reaction. After the first reaction the valve returns to its contact with the collar, and the mucilage secreted by the glands seals the seam more or less perfectly. The quadrifid hairs begin to absorb the liquid from the lumen, either forcing it eventually into the vascular system, or else to the exterior through the two-celled glands of the external wall. Since the semipermeable membrane covering the bladder allows the penetration of water slowly or hardly at all, and the valve seals up the aperture, water cannot enter fast enough to counter-balance the loss occasioned by the activity of the quadrifids, and the lumen slowly loses its contents. As a result, the lateral walls are forced inward, which may be effected either by the combined external hydrostatic and atmospheric pressure, by the tension of the water within, or by both acting together. In this new position the walls are under strain, caused by the deformation of their com-



ponent turgid cells, and perhaps in part by the compression of the air bubbles in the intercellular spaces, and the pressure within the bladder becomes negative in respect to the medium. Displaced inward by the compressed walls, the bowed valve is pressed more strongly against the collar, the better to resist the growing pressure from without, and between these two forces it is in unstable equilibrium. If now a small copepod collides with one of the four bristles, the latter in virtue of its leverage deforms the rim of the valve, breaking the contact between it and the collar, and allowing free communication between the external and internal fluids. Free to expand, the lateral walls snap apart, sucking in a current of water through the aperture, and along with the stream the devoted crustacean. Should the animal seek to escape by the door which afforded such ready entry, he will find it tightly barred. The whole process occurs in the wink of an eye, and one who has witnessed it does not wonder that so many of the earlier naturalists failed to observe what actually does occur.

If the above explanation of the process is the true one, several consequences ought logically to follow, and these remain to be considered. Suppose that a bladder is not disturbed by an animal for a period much longer than that required for its complete setting, will it fire automatically from over-tension, or will the several processes involved in its setting reach a steady-rate? Merl investigated this question, but because of experimental difficulties could form no definite conclusion. In default of direct observation on normally conditioned bladders, we must rely upon the results obtained by immersing bladders in a hypertonic solution of a substance to which the membrane is impermeable. Such experiments show that after the tension has exceeded a certain limit, the system becomes more stable as the tension increases. In pond water there is probably always some infiltration of water through the walls, and this would naturally increase as the pressure gradient becomes sharper. At the same time the rate of absorption by the quadrifids must be reduced because of the greater resistance opposed to their action, and the two processes should approach each other in intensity and establish a steady-rate. The tension in the lumen can hardly, under natural conditions, exceed in numerical value the osmotic pressure of the cell-sap of the quadrifids. The presence of air-bubbles in bladders which are functional is in no way incompatible with Czaja's theory, since by the expansion and rarefaction of these the internal pressure may be reduced very considerably. It is not known

to what extent this must be reduced in order that a reaction may occur. Withycombe was able to detect an increase in volume of gas bubbles contained in the lumen during the setting of the bladder, but unfortunately he gives no measurements.

The frequent discharge of bladders while passing from water into air is probably caused by the pull exerted on the bristles of the valve by the contracting surface film, which succeeds in deforming the valve.

A more serious difficulty lies in the infinitesimal force of impact apparently sufficient to produce the reaction. In Hegner's<sup>(21)</sup> experiments, paramoecia were caught with such frequency that it appears highly probable that the trap can be sprung by a minute protozoan impinging against some portion of the valve, presumably the bristles, although this was not actually observed. Ekambaram<sup>(13)</sup>, by means of an ingenious device, tried to evaluate the force necessary to push inward the valve of a transversely halved bladder of his undetermined species. He records only two measurements, which, however, agree closely, and found the required weight to be 250 and 280 mg., respectively. He believes that many of the animals caught by the bladder could not exert a force of this magnitude. Had the valves used in his experiments been part of a set bladder, a portion at least of this force would have been supplied by the excess pressure of the medium, and a smaller weight would have sufficed to spring the trap. Withycombe observed that the edge of the valve fits into a groove in the collar, and believed the only possible movement which could free it to be an upward one resulting from a contraction of the valve cells. He was of the opinion that the principal motor tissue is situated at the base of the four bristles, and contracts only as a result of the stimulation of these appendages, drawing the valve out of the furrow and allowing it to be forced inward by the excess pressure of the medium. Ekambaram<sup>(14)</sup>, in 1924, still persisted in believing that these hairs are sensory perceptors, and the valve a motor tissue which reacts through changes in turgescence.

The idea that the bristles are sensory arose from their resemblance to the sensory hairs of *Dionaea* and has been persistent since the time of Darwin, who rejected it. The fact that attempts to produce rigor have been apparently unsuccessful does not really invalidate the view that sensorimotor phenomena are normally responsible for the reaction, in the manner held by Withycombe. The essential part of the reaction is the dilation of the bladder, and it is altogether conceivable that, while this expansion normally is released by a con-

traction of the valve following slight stimulation of the perceptor bristles, the application to the valve, made insensitive by any means, of a pressure strong enough to tear it away from the collar and allow its inward movement, would produce the same end result. A box-trap, such as a boy sets to catch rabbits, which responds to the slightest touch on the trigger, may also be sprung by a stronger force applied directly to the sliding door. Apparently Merl and Czaja failed to appreciate the necessity to distinguish between these two possible modes of securing the reaction. On the other hand, the failure to secure response by any form of stimulation other than the mechanical weighs strongly against the assumption of irritability, since, as remarked above, this would be the unique instance of such strictly limited sensitivity, and at present it seems safest not to accept the view which favours it. It is certain that the activity of the bladder is not contingent on its irritability; and if it is ever demonstrated that it does possess a motor-tissue, this will probably come to be considered as an added refinement creating greater delicacy and increasing the number and kinds of prey captured, rather than an essential part of the mechanism.

#### V. THE MEMBRANE OF THE BLADDER

The very extensive special investigations of Czaja(8,9) on the nature of the membrane enclosing the bladder can unfortunately receive only the briefest mention in an article with the scope of the present, and for fuller details the reader must be referred to the original memoirs. Since the properties of the membrane are of such importance to the proper functioning of the bladder, and to the interpretation of experiments attempting to produce rigor, a few of Czaja's main conclusions are repeated here.

The experimental procedure employed was simple. Fresh, active bladders of *U. vulgaris* or *U. neglecta* were transferred in the discharged condition to the desired solution, all water adhering to the exterior having been previously removed with filter-paper, to avoid dilution. The bladders were allowed to remain in the experimental solution for 15 minutes, which normally is sufficient for them to regain the set condition, and after this period their activity was tested by touching the valve with a needle. It was found that when bladders were placed in graded concentrations of a solution of a single substance, the force which it was necessary to apply to the valve to secure a reaction increased with the concentration, until finally the bladder could not be caused to react even by applying a strong

pressure. The highest concentration at which the dilation of the bladder could be secured by exerting a "strong pressure" on the valve was taken as the concentration limiting to the activity of the bladder. It was recognised that the criterion employed was in a measure subjective, but the use of a considerable number of bladders in each experiment somewhat modified the likelihood of an error in interpretation. After probing the activity of a bladder, it was returned to distilled water and its subsequent fate observed after 24 hours.

The number of substances tested in this manner was particularly large, comprising inorganic neutral salts, mineral acids and bases, organic acids, alcohols, narcotics, aldehydes, alkaloids, amides, etc. In general, these chemicals agreed in causing loss of function and irreversible injury when applied above a certain concentration. Opposed to these in their effects were a number of "indifferent" substances, including glycerine, sucrose, glucose, mannite, asparagin, glycocoll and the like. Chemicals of the first class, in concentrations below the limiting, caused no injury to the bladders, or if injurious effects were evident they were in general of a reversible nature. Above the limiting concentration, the bladders became completely unresponsive, and after the period of 24 hours allowed for their recovery were greatly distended and evidently dead or dying. A pronounced exception to this behaviour was found in mercuric chloride, which caused permanent injury in concentrations below the limiting, and the hydroxides of sodium and potassium, which did not cause irreversible injury in concentrations well above the limiting. The indifferent substances caused loss of function only when their concentration was so great that the over-tension produced in the bladder made the release of the valve impracticable, in the manner indicated in the previous section. Returning the bladder to distilled water was followed by complete recovery, unless it had been collapsed by an extremely high osmotic pressure. The appropriate micro-chemical tests failed to give evidence of the penetration into the bladder of any substance of either class from solutions more dilute than the limiting concentration, although above this concentration penetration was detected.

Czaja supposed that the bladder is covered with a cuticula in which is dispersed a hydrophil gel. The adsorption of ions by the disperse phase causes it to become more continuous, at the same time diminishing its permeability to water, and with a sufficient concentration of the electrolyte it forms an almost perfect barrier

to the penetration of aqueous molecules. The degree of tension attained by the bladder is the resultant of two antagonistic processes, the removal of the water from the lumen by the quadrifids, and its penetration through the walls from the medium to the interior. Since the value of the second rate is reduced by subjecting the membrane to the action of an electrolyte, the tension is built up more quickly and reaches a greater maximum than is possible in pond water. The over-tension makes it necessary to exert a greater force to release the valve (see p. 280) and thereby decreases the apparent sensitivity of the bladder. The effect is at least roughly quantitative, and with increasing concentrations a greater and greater force is necessary to secure a reaction. If the concentration is below the limiting, only the superficial layers of the membrane are affected, and the normal permeability may be regained by placing the bladder in pure water, where the active ions diffuse out of the gel. The coagulation of the gel stops the further penetration of the electrolyte causing it, so that the latter cannot reach and injure the protoplasm of the wall cells. Excessive concentrations, instead of producing a fine superficial condensation, throw the whole colloid into a coarse, porous coagulum, which is permeable both to water and to the chemical in question. Since the penetration of water can proceed with small obstruction, it is impossible for the quadrifids to build up the tension necessary for the reaction, and the access of the chemical injures or destroys the protoplasm. The changes caused by concentrations above the limiting are for this reason in general irreversible. The injurious non-electrolytes have a similar action on the membrane, but by virtue of different properties.

Indifferent substances do not change the condition of the membrane, which is *ab initio* impermeable to them, and their action is purely osmotic, and is reversible.

Placing bladders collected from natural waters into distilled water caused them to become more sensitive; they reacted to slighter pressure. This is explained by supposing that the small amounts of electrolyte adsorbed by the gel from the original medium diffuse out into the distilled water, which results in greater dispersion, and consequently in increased permeability of the membrane. The tension of the bladder cannot become so strong as previously, the valve is not held so tightly and may be released by a weaker contact. Bladders which were allowed to remain for a long time in distilled water showed plasmolysis by much lower concentrations of  $\text{KNO}_3$  than were necessary to plasmolyse fresh bladders, an indication of the increased

permeability of the former. Thus, fresh bladders showed plasmolysis after 15 minutes in 3 *M* KNO<sub>3</sub> but not in 2 *M*. After 27 days in distilled water plasmolysis was obtained after the same interval of treatment by employing *M*/2 KNO<sub>3</sub>, and intermediate periods produced corresponding reductions in the necessary concentration. On the other hand, that an electrolyte decreases the permeability of the membrane was demonstrated by placing in 3 *M* glycerine solution bladders previously treated for 15 minutes with *M*/1 to *M*/16 KNO<sub>3</sub>. The glycerine was unable to withdraw water from the lumina of such bladders by diosmosis, they showed no over-tension and could be sprung after 1 hour 15 minutes in the glycerine, while the walls of the control became very concave, and the bladders could not be made to react.

#### VI. THE NUMBER AND KIND OF PREY; DIGESTION; ABSORPTION; IMPORTANCE OF THE CARNIVOROUS HABIT TO THE PLANT

A comparison of the accounts of all observers, in whatever country, indicates that by far the most common prey of the aquatic bladder-worts are small entomostracan crustacea, principally of the Copepoda (*Cyclops*), Ostracoda (*Cypris*) and Cladocera (*Daphnia*). Of the Malacostraca, the fresh-water amphipod *Gammarus pulex* has been reported to occur sparingly in the bladders (Garbini<sup>(15)</sup>, quoted by Brumpt<sup>(3)</sup>). Protozoa, including rhizopods, ciliates and flagellates, are often captured in considerable numbers, and the occurrence of green flagellates has been recorded. The aquatic larvae of insects, which are often large and conspicuous, have been seen in the bladders by many naturalists, and must be recognised as common victims. Larvae probably of the mosquito were found by Mrs Treat and by Darwin, in 1875, and since that time they have repeatedly been observed within the bladders. Nematodes and rotifers were found within the bladders of *U. neglecta* by Garbini, and of *U. vulgaris* by Hegner. Moseley<sup>(29)</sup> describes the capture of newly-hatched roach by *U. vulgaris*, observed at Oxford by G. E. Simms. These fish are too large to be wholly engulfed, but were held in the bladder usually by the head, although often by the tail or the still-attached yolk-sac, the rest of the body protruding through the orifice. A few examples were caught with the head in one bladder and the tail in another, the body forming a bridge between the two. Young tadpoles are also occasional victims of the bladders (Gräbner<sup>(19)</sup>, Fig. 135). There is probably no species of aquatic animal sufficiently small to be ingested which is not occasionally a sacrifice to the

carnivorous habit of the plant, and, as is to be inferred from the method in which capture is effected, no selection of organisms on the part of the bladder has been recorded. The relative abundance of different species among the prey is probably determined almost wholly by the size, habits, activity and density in the medium of the various organisms. In addition to animal booty, small algae, such as diatoms, blue-greens and desmids, are often observed within the bladder.

The consideration of the quantity in which the prey are captured is of importance in forming an estimate of the value of this form of heterotrophic nutrition to the plant. Statistical observations made upon plants taken from natural habitats are of most value in this connection, but the frequently made determinations of the rate of entry of small animals into empty bladders placed in well-populated water are also of great interest. Garbini<sup>(15)</sup> investigated the contents of 610 bladders of *U. neglecta*. Of these 62 were empty, 44 contained unrecognisable debris, while the remaining 504 contained various organisms, to the number of 2084, or an average of about 4 to the bladder. Four species alone accounted for 1550 individuals, or three-quarters of the entire catch; these were *Stilonychia mytilus* Ehrbg. (a protozoan) 195, *Chydorus sphaericus* D. F. Müller (a cladoceran) 872, *Monomatta longiseta* Bartsch (a rotifer) 185, and *Cyclops signatus* Koch 298. It is probably a general rule that when a large number of animals is trapped, one or a few species will constitute the great majority, while the remaining species captured will have only a scattering representation. Hegner gives figures indicating the total number of organisms falling prey to a single large plant. He estimated from a partial count that a branch consisting of a main stem 110 cm. long, and bearing 4 side branches with a combined length of 110 cm. supported approximately 13,860 bladders. In 10 bladders selected at random, the number of *Entomostraca* which had been captured ranged from 6 to 22, with an average of 12 per bladder. On a conservative estimate, the bladders of this portion of the plant contained about 150,000 *Entomostraca*, in addition to numerous animals of other classes. Since the victims eventually break down and disintegrate, while new ones are constantly captured by the same bladders, the total number of organisms trapped by a single favourably situated plant during a summer must be enormous. In other material, Hegner found 512 euglenas in a single bladder, and 10 bladders examined contained an average of 215 of these flagellates each.



Of observations regarding the rapidity with which animals are captured, those of Bűsgen<sup>(4)</sup> and Brumpt<sup>(3)</sup> are of the most interest. The former placed a spray of *U. vulgaris*, bearing empty bladders, into a vessel of water teeming with the cladoceran *Chydorus*. Within 1.5 hours, the bladders examined had captured on the average 3 animals each, whence Bűsgen estimated that the entire specimen, 15 cm. long, and with 15 developed leaves, must have engulfed no less than 270 of these animals. A single bladder had accounted for 12 individuals, or an average of one every 8 minutes. Brumpt's experiments demonstrate the completeness with which the fauna of a given volume of water may be utilised by the bladders. He placed 100 larvae of *Anopheles maculipennis*, 1.5 mm. long, into a vessel containing 2 young branches of *U. vulgaris*, and in less than 3 hours almost half were taken. When the experiment was repeated, using *Culex apicalis* instead of *Anopheles*, 50 per cent. were caught within the first hour. Hegner<sup>(21)</sup> found that 30 per cent. of the bladders immersed in *Paramoecium* cultures succeeded in capturing one or more individuals within an hour.

As perhaps best exemplified by the observations of Simms (Moseley<sup>(29)</sup>) mentioned above, the bladders may often capture animals considerably larger than themselves. The mosquito larvae caught in the bladders may be almost 1 cm. in length, in which case they are coiled up within the lumen, and a larva 7.3 mm. long was found in a bladder 3 mm. in length. Mrs Treat observed that the entry into the bladder of such a large larva required in one case between 3 and 4 hours. Darwin also noticed larvae which were half within, and half outside the bladder. According to Brumpt, the larvae which came under his observation were usually held with either one or more of the posterior segments or the entire body except the head within the bladder, but the head always protruding, indicating that they had been drawn in with the posterior end foremost. The posterior end, which bears the anal brushes, is very actively moved while the animal swims, and for this reason probably often beats against the valve and releases it. Ostracods 0.6 mm. long and 0.4 mm. broad easily enter the large bladders of *U. vulgaris americana*.

Do the bladders of *Utricularia*, like the pitchers of *Sarracenia* and *Cephalotus*, possess any provision to lure on the prey they are to capture? Several adaptations for the attraction of aquatic animals have been pointed out by various naturalists, some of which are, to say the least, fanciful. Bűsgen observed that small aquatic animals are attracted by vegetable mucilage, and believes that the slime



secreted by the numerous glands surrounding the aperture serves to attract the prey. Mrs Treat observed larvae feeding on the long hairs at the orifice. Von Luetzelburg demonstrated the presence of sugar in the collar and the lower margin of the valve, and believed that this, along with the slime, attracts small organisms. Darwin surmised that the spot of light reflected from the convex surface of the valve might serve as a lure.

Several, but not all, of the purely terrestrial insectivorous plants secrete proteolytic enzymes which effect the digestion of their prey. This seems clear in the cases of *Dionaea*, *Drosera*, *Drosophyllum*, *Nepenthes* and *Pinguicula*. On the other hand, *Sarracenia* and *Cephalotus* lack the faculty of producing such secretions, and certain genera, such as *Genlisea*, *Polypomphyolux* and *Aldrovanda*, still await investigation. Darwin could discover no digestion of the small cubes of roast meat, albumen and cartilage which he inserted into the bladders of *Utricularia*, and concluded that no proteolytic enzyme was present there. Later Büsgen<sup>(4)</sup> and Goebel<sup>(18)</sup> announced their confirmation of Darwin's conclusions.

The period necessary to bring about the death of an imprisoned animal ought to afford some suggestion of the intensity of the action of possible digestive enzymes, or of the presence of any poisons. Mrs Treat observed that larvae remained alive within the bladders for 24–36 hours. Cohn found that captured animals occasionally lived for 6 days, and records that the larva of a fly, after swimming around in the bladder for 3 days, finally made good its escape by eating a hole through the wall. Büsgen believed that the bladders are protected from such injury, inflicted either from within or without, by the presence of tannin in the cells, which renders them distasteful to animals. He observed that a piece of tissue previously treated with alcohol or hot water, to remove the tannin, is readily attacked by cyprids, while a fresh piece is avoided. He found that animals captured in the bladders often died within 24 hours, while others, which had become motionless after this period of captivity, revived when removed to fresh water. From this behaviour he inferred that suffocation was perhaps responsible for the death of the victim. This mode of death has been suggested by other authors, and the question of asphyxiation within the bladders requires further investigation, especially in the light of Hegner's results. However, all of these fragmentary observations show that death does not always rapidly supervene, but the animals may live a considerable time in such captivity. The more careful experiments of

von Luetzelburg<sup>(26)</sup> indicated that the contents of the bladders exert some deleterious effect upon the captured organisms, even when removed from the lumen, but he did not distinguish between the fluids of the cavity and those present in the cells of the wall. By grinding whole bladders in a mortar and extracting with glycerine, he secured a solution in which larvae and small crustacea showed signs of approaching death after 11 hours. In the control (glycerine water) they behaved much as in their normal medium. Placed in this fluid, flies swam on their sides after 7 hours, but recovered upon removal.

Most of the scanty observations available seem designed to determine the longest period of imprisonment an animal can survive, rather than the shortest interval necessary to destroy it. Long periods of persistence may indicate merely an old or otherwise languishing bladder. The careful researches of Hegner<sup>(21)</sup> on the fate of captured protista are free from this criticism. Hegner found that euglenas not only remained alive indefinitely within the bladders, but actually multiplied there. *Phacus longicaudus*, another green flagellate, also lives and may multiply within the bladders; captured specimens of *Heteronema acus*, although not killed during confinement, were not observed to increase in numbers. On the other hand, captured paramoecia died in an average time of 75 minutes after their entry, and their disintegration usually followed rapidly. If, instead of being captured in the normal manner, the paramoecia were inoculated into a bladder by a pipette, their period of activity, although it still varied greatly, was generally considerably lengthened, and in certain cases reached 17 days, but in others death supervened after 25 minutes. If the bladder had been previously irrigated by sucking out the contents with a fine pipette, animals captured or inoculated into it generally died within 2 hours, although here again great variation was evident. In old, dead bladders, or in bladders killed by heat, paramoecia did not die within a short time, and often eventually made their escape. When placed in liquid withdrawn from the bladder cavity, paramoecia were not killed even after 48 hours, and the result was the same whether the bladders which supplied the liquid had previously killed paramoecia, or had not been infected by them. *Stentor polymorphus*, *Colpidium colpoda* and *Stylonychia pustulata* were all killed when impounded in the bladders, but *Centropyxis aculeata*, a shelled rhizopod, might live a week, and apparently died only from starvation. These experiments raise many interesting questions, the answers to which must await future investigation. Why should the green flagellates *Euglena*, *Phacus* and

*Heteronema*, and the rhizopod *Centropyxis* remain unharmed within the bladders, while the colourless infusoria *Paramoecium*, *Stentor* and *Stylonychia* are soon killed? Why should paramoecia die after a short period within the bladder, but remain alive for 48 hours and more in the same liquid when removed from the bladder? Do these results indicate that the substance which causes death is not always present in effective concentration in the lumen, but is secreted there when a captured animal gives the proper stimulus? Is suffocation a factor in bringing on the death of the prey?

Von Luetzelburg reinvestigated the problem of the occurrence of digestive enzymes within the bladder, with results contrary to those of Darwin, Büsgen and Goebel. He placed small, angular particles of egg albumen and cheese in an extract made as described above (p. 290) and after three days the edges had lost their sharpness, and showed signs of corrosion not evident in the control. Neither when these proteins were used, nor with raw or cooked flesh, fibrin, milk, etc., could he secure a positive biuret reaction of the solution after 8 hours. Although no disinfectant was used, no odour of indol or skatol became evident in the solutions, and even after 27 days under conditions favourable for bacterial growth, no evolution of bacteria or moulds occurred. Drops of this extract liquefied gelatin within 4 days. The behaviour of a pure extract, made without the addition of glycerine or other substance, was next investigated. Cubes of albumen and cheese subjected to its action gave signs of corrosion as in the glycerine extract, and oil globules gave evidence of saponification after 8 hours. Yet all of the common tests failed to give a clear indication of the presence of soluble protein derivatives in the fluid. Finally von Luetzelburg was able to demonstrate the digestion of casein in the following manner: to 30 c.c. of bladder extract he added 50 c.c. of a suspension of casein containing 1 per cent. of  $\text{Na}_2\text{CO}_3$ . After neutralisation, the addition of 1 per cent. of ethyl alcohol caused the precipitation of the protein. The bladder extract was allowed to act upon a similar, unprecipitated mixture, and after 13 hours the addition of alcohol no longer caused a precipitate, indicating that the casein, which had originally formed the precipitate, had been digested. In this experiment, which was repeated 10 times with the same result, several drops of ether or chloroform were added to the solution to suppress bacteria. The tryptic enzyme present in the sap must be either very inactive or very dilute, considering the long period required for it to produce results even in such great concentration of the bladder fluid.

The conspicuous absence of the odours resulting from putrefactive decay in solutions containing protein substances, and the failure of bacteria or moulds to thrive in them under conditions apparently proper to their development, was rather surprising, and required further investigation. The lumina of young, still-fasting bladders seemed wholly free of bacteria, and showed no evolution of them when filled with sterile culture medium introduced by a pipette, under aseptic precautions. The surface of bladders was sterilised by treatment with  $\text{HgCl}_2$  1 : 1000, water and alcohol, and the fluid then pressed out of the cavity and allowed to drop on a gelatine plate, using all precautions to prevent contamination. Here only a very meagre and sickly culture of bacteria resulted from the inoculation. Von Luetzelburg concluded that bacteria do not normally produce the decomposition of captured animals, their presence is merely incidental, and their growth is checked by some deleterious substance produced by the bladder. Only injured or old bladders contain many bacteria and protozoa. Goebel(18) had previously demonstrated that the fluid in the pitchers of *Cephalotus* contains some substance which prevents the putrefaction of the captured insects, and had already suggested, as a result of some fragmentary experiments, that the bladders of *Utricularia* might be found to possess a similar peculiarity. Von Luetzelburg was able to demonstrate conclusively the presence of benzoic acid within the bladders, and this is the agent which prevents the evolution of the bacteria responsible for putrefaction. He obtained crystals of this acid, determined its melting point and crystalline form, and studied its physiological action on moulds and bacteria. Benzoic acid is present also in the leaves of the related *Pinguicula vulgaris*. The presence of such a disinfectant in the bladders is important, since indol and skatol are toxic to them even in great dilution.

Although unable to demonstrate the action of proteases upon the ingested food, Darwin undertook to show that the substances derived from what he believed to be the bacterial decay of captured animals were absorbed and utilised by the plant. He observed that in completely empty bladders the protoplasm in the arms of the quadrifid hairs is clear and transparent, except for a small, more highly refractive body which he took to be a "modified nucleus," but which Goebel(18) demonstrated to be a small crystal of calcium oxalate. After the bladders have made a capture, the appearance of the quadrifids changes. The protoplasm now becomes yellowish and often shrunken, and contains numerous, highly refractive, yellowish

granules. This granulation is similar to that which he had observed in the tentacles of *Drosera* and the glands of *Dionaea* upon the absorption of food, and naturally he concluded that it indicated the absorption of nitrogenous substances in this case also. He found that he could induce a similar condition of the quadrifids by placing halved bladders in solutions of  $\text{NH}_4\text{NO}_3$ ,  $(\text{NH}_4)_2\text{CO}_3$ , urea, meat extract and putrid infusions of raw meat, but gum arabic and sugar solutions were inactive. Similar but more pronounced alterations of the protoplasm occurred in the glands surrounding the orifice of the bladder and finally led to the death of the cells. This observation, coupled with what he thought to be the irreversible nature of the granulation of the protoplasm, raised the question of whether the phenomena which he observed might not indicate injury to the protoplasm rather than the absorption of food. The inquiry was taken up at a much later date by von Luetzelburg, who showed that a very similar appearance in the protoplasm could be induced by such non-nutritious chemicals as  $\text{ZnSO}_4$ ,  $\text{PbCO}_3$ ,  $\text{MnSO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$  and  $\text{HgCl}_2$ , and by extremely dilute solutions of indol or skatol, and therefore granulation is not conclusive evidence of the absorption of food. He further showed that such phenomena as Darwin had recorded result from the presence of an excess of readily digestible food, but that they could be reversed if the food is removed as soon as the granulation of the protoplasm becomes evident, and the bladder washed with sterile water. If the substance is allowed to remain in the bladder for 1 or 2 days longer, the granulation is not reversible, and leads unconditionally to the death of the hairs and eventually of the entire bladder.

Goebel (18) showed that the granules present in the bladders which he studied were not proteins, but globules of oil, probably the lecithin which oozes from the bodies of the dead crustaceans. He assumes that this is a source of the fat stored in the winter bud in the autumn.

Certain changes which occur in the bladder, and in the leaf of which it is a part, upon feeding the former with nutrient solutions, furnish excellent evidence that the proffered food is absorbed and promotes the growth of the plant. If asparagin, albumen or flesh-extract is introduced into a bladder by means of a fine pipette, an increased production of chlorophyll is manifest throughout the whole bladder, and all portions of the antennae, even those normally colourless, become intensely green. The whole bladder experiences an increase in size which in *U. minor* may amount in 4 days to one-third of the original dimensions. In *U. vulgaris* giant bladders,

6.2 mm. long, were produced by this artificial feeding. In addition, the antennae become greatly swollen, especially at their bases, and the whole bladder assumes a monstrous appearance (see von Luetzelburg (26), Figs. 7 and 8, p. 169). A great number of adventive shoots arise from leaves bearing bladders fed in this manner and in one instance 19 shoots sprang from a single leaf. However, Czaja (7) found that when bladder-bearing leaves were kept for a long time in distilled water, the bladders died and the leaves gave rise to adventive shoots, so that the production of such growths cannot in itself be taken as a criterion of favourable nutrition. In addition "double-bladders," or rather two single bladders arising from the same stalk, spring from such artificially fed leaves, and the leaf apex may be stimulated to produce a bladder. These results occur even when the solutions used ultimately bring about the death of the bladder, provided that the food is not supplied in excessive concentration.

The best method of securing information concerning the value or necessity of an animal diet to a carnivorous plant is to grow two parallel series of cultures, one of which is provided with flesh, the other deprived of it, and to compare the vegetative and reproductive activity, as expressed in growth and the production of seed, of the two lots. This approach to the problem has been used with cogent results in the case of *Drosera rotundifolia* by Büsgen, Francis Darwin, and Kellerman and von Raumer (see Goebel (18), p. 207), proving convincingly the value of a carnivorous diet. The results of similar experiments with *U. vulgaris*, carried out by Büsgen (4), in which vegetative growth alone was measured, are less convincing only because of the very small number of plants used with success. Büsgen started his cultures with the apical portions of shoots which he cut off just above the youngest leaf-bearing bladders which had captured prey. All cultures were supplied with water from the same source, which contained numerous animals on the one hand, and had been strained to remove the larger organisms on the other. Most of the experiments inaugurated had to be discontinued because the plants thrived so badly, from unfavourable weather or other reasons. The results of the series which gave the clearest results are presented here:

	Length July 4 cm.	Length July 30 cm.	Growth in 26 days cm.	
Plant <i>a</i> , fed in greenhouse	14.5	47.0	32.5	} Mean 21.8 cm.
Plant <i>b</i> , fed in greenhouse	9.0	20.0	11.0	
Plant <i>a</i> , unfed in greenhouse	13.0	28.5	15.5	} Mean 10.5 cm.
Plant <i>β</i> , unfed in greenhouse	11.0	16.5	5.5	

The fed plants also produced more leaves than the unfed. Other shoots were kept in vessels placed in a manured hot-bed. Here the fed plants grew 60 cm., as opposed to the growth increment of 30.4 cm. made by the unfed plants. The average increment in length of the fed plants was twice that of the unfed, in both situations. In another series the fed plants grew well, while most of the unfed soon stopped growing and produced winter buds, so that no direct comparison could be made between the two cultures. However, the unseasonable production of turions is itself an indication of conditions unfavourable to growth (Glück(16), Goebel(18)). Goebel (18), p. 206) "saw years ago, in De Bary's laboratory at Strassburg, unfed plants which in size lagged immensely behind those which were fed, but otherwise under the same conditions."

In conclusion, the criticism of Langeron(25) that "en réalité, nous ne savons rien sur la fonction des vésicules des utriculaires," seems to the present writer to be hardly justified. Langeron adduces in support of his contention cases in which plants were grown from winter bud to winter bud in water containing little plankton. The presence of the turion with its large supply of stored food renders the plant for a time independent of outside sources of nutriment, and introduces a complicating factor hard to evaluate; the production of the second winter bud is likely to ensue the faster the more unfavourable the external conditions become. Undoubtedly over-feeding causes the injury or death of the bladder, but similar unfavourable results follow the capture by *Drosera* of very large insects, because they are too bulky to become thoroughly impregnated with formic acid and digestive enzymes before putrefactive decay sets in. Small animals, captured a few at a time and slowly killed and digested, supply an abundance of nitrogenous food, and the result of their ingestion is not so harmful. The bladders do not normally remain functional throughout the vegetative season, and it is probably true that the capture of animals somewhat shortens their period of activity, if only because of the accumulation of unassimilable debris, although no direct experiments on this point, other than those of von Luetzelburg and of Darwin on artificial feeding, are known to the writer. However, even in the latter case, before dying the bladders pass on to the leaf valuable food substances which cause more rapid growth. Other organs, which absorb substances essential to the welfare of a plant, or manufacture its food, suffer a similar fate; the root-hair is at best a very transient structure, and the leaf of an evergreen or a tropical plant, over a long period of its activity,

accumulates substances which interfere with its functions and cause its death. They are not for this reason considered as without advantage to the plant. Excess feeding under artificial conditions causes abnormal and monstrous growths, but these have not been reported as occurring under natural conditions, where the supply of food is more uniform and in a form less rapidly available. The fact that other species, not carnivorous in habit, can thrive side by side with a carnivorous species affords no good evidence that animal food is not advantageous or even essential to the latter. The two species may merely have solved in different ways the problem of surviving in the (to most plants) unfavourable environment of a bog or marsh, the one proclaiming itself by an obvious structural modification, the other hidden in occult physiological changes. It is also possible that *Utricularia* is a facultative rather than an obligate carnivore, and the fact that it may be placed in an environment in which it can thrive without animal food affords no proof that in another habitat the organic diet is not indispensable<sup>1</sup>.

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<sup>1</sup> Our discussion in the present paper has been devoted exclusively to the aquatic species of *Utricularia*. The terrestrial and epiphytic species differ so conspicuously both in their conditions of life and in the structure of the bladders that it is hardly safe to apply to them any of the conclusions reached from a study of the aquatic species. Practically no work has been done on their physiology, and the observations on the prey captured by them are not nearly so satisfactory as in the case of the more familiar floating species (see Schimper (32)).



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## THE INTER-RELATIONSHIPS OF THE ARCHIMYCETES (*concluded from p. 260*)

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(With Plates VI to VIII, III text-figures and 8 diagrams in the text;  
the Plates, 67 text-figures and 5 diagrams in Part I)

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### 7. ANCYLISTACEAE

Sharply separated from the Chytridiales by Schroeter(119), but less markedly so by later workers, are the Ancylistaceae and the Lagenidiaceae. The members of these groups attack green algae belonging to the Conjugatae. Apparently the chief point of distinction between the Chytridiales and the Ancylistaceae lies in the fact that the genera belonging to the latter exhibit marked sexuality with well-differentiated sex organs. The zoospores, moreover, differ from those of the Chytridiales in having two laterally placed flagella. Butler among others is of the opinion that the number of flagella offers a fundamental distinction between the Chytridiales and the Ancylistaceae. Atkinson(9) however is inclined to the view that a difference in the number of flagella does not prevent them forming a phylogenetic series. As he points out, biflagellated zoospores are sometimes found in zoosporangia of genera usually possessing only one, and, conversely, that zoospores normally possessing a single flagellum are found in species which usually have two. He suggests that in the genera *Woronina*, *Pseudolpidium*, *Woroninella* and *Rozella* the formation of two flagella has been evolved from forms which originally possessed only one.

Very likely the whole question is bound up to some extent with the gradual evolution of diplanetism. We find such a condition appearing in *Olpidiopsis* where the zoospores of some species swim about for from two to five minutes with equal but oppositely directed

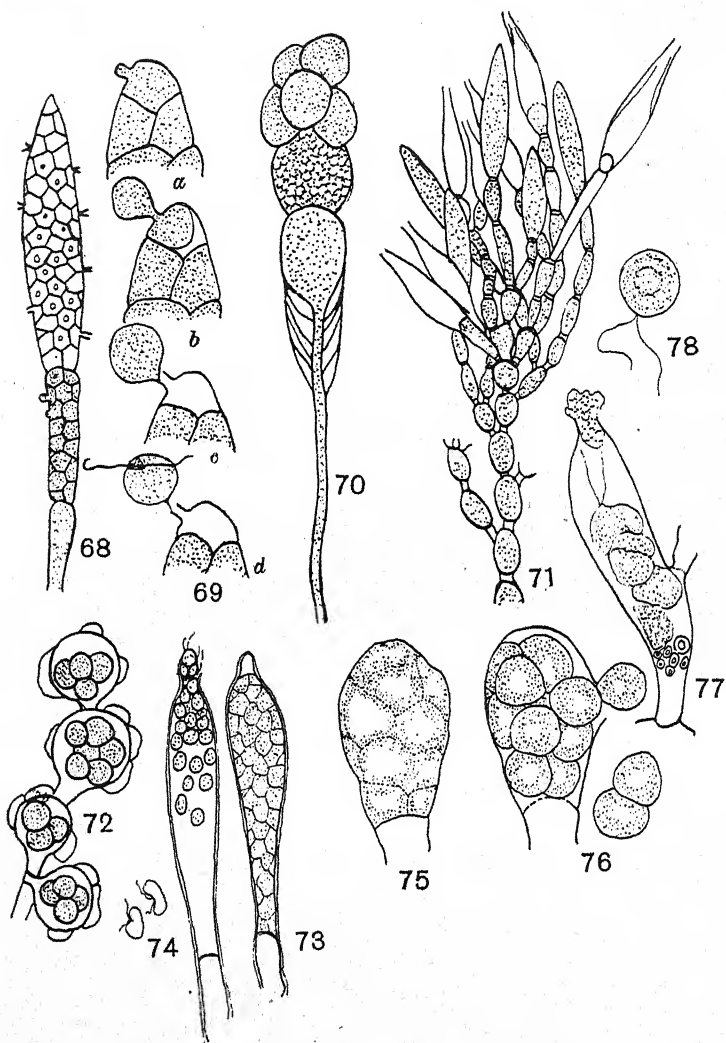
flagella, though in other species there is but a single flagellum. They then rest for about ten minutes and the flagella are withdrawn. Afterwards, fresh active movement again occurs. In *Harpochytrium* the zoospores show amoeboid movement while still within the zoosporangium, but once they have emerged, they continue swimming until a suitable habitat is found. They may remain active for as long as six hours.

In the genus *Lagenidium* we find an example of diplanetism. In *Lagenidium pygmaeum* the zoospores are differentiated within the prosperangium. They migrate separately through the exit tube and become aggregated in a mass within its swollen end whence they finally escape and pass through a second swarming period. In *Myzocyttium proliferum* the protoplast becomes segmented into a number of parts corresponding to the number of the zoospores, but their differentiation does not take place until they have passed into the sporangium. Atkinson(8) describes the condition in another species of *Lagenidium* in which the whole sporangium whose zoospores are being differentiated floats off, at first showing plastic movement only. Later, flagella develop, and the whole structure swims about like a *Pandorina* colony before the zoospores finally separate.

Zopf(156) cites a case of diplanetism in *Rhizidiomyces apophysatus*, in which the protoplasm passes out along a much elongated exit tube not as a continuous stream, but as distinct masses, each of which forms a separate zoospore. He suggests that this indicates a relationship between the Rhizidiaceae and the Ancylistaceae.

It is in the Saprolegniaceae and the Pythiaceae, however, that the best and most complete examples of diplanetism are to be found.

In *Pythiopsis* the zoospores are oval with two apical flagella, they swim about and then settle down and produce a fresh plant. In the more advanced genus *Saprolegnia* the zoospores follow the same course of development, but after liberation of zoospores with paired apical flagella, they round themselves off and become encysted. Later the contents emerge again, but the fresh zoospores can be distinguished by their shape and by the presence of two laterally placed flagella. In the genus *Achlya*, the first part of the process is reduced. The zoospores are active while within the zoosporangia, then they become passive while arranged round the mouth, and again become active when they have escaped from the vicinity of the zoosporangium. In *Aphanomyces*, the first stage is even more



Text-figs. 68-78. Saprolegniales

68. *Dictyuchus* sp.; two zoosporangia showing the net structure, the lower one still containing spores. (After Weston.)
69. *Dictyuchus* sp.; a-d, stages in the emergence of the zoospores from the sporangium. (After Weston.)
70. *Myrioblepharis paradoxa*; multiciliate zoospores of an older sporangium at the apex of a new one. (After Thaxter.)
71. *Gonapodya siliquaeformis*; a portion of a plant showing the constrictions, and renewal of sporangia by proliferation. (After Thaxter.)
72. *Saprolegnia anisospora*; oogonia in chains. (After Coker.)
73. *Saprolegnia* sp.; zoospore formation.
74. *Saprolegnia* sp.; two escaped zoospores.
75. *Thraustotheca clavata*; a mature sporangium. (After Weston.)
76. *Thraustotheca clavata*; escape of sporangiospores. (After Weston.)
77. *Monoblepharis brachyandra*; zoosporangium with biciliated zoospore escaping. (After Thaxter.)
78. *Monoblepharis brachyandra*; biciliated zoospore. (After Thaxter.)

reduced, and no flagella are formed, the zoospores being represented only by elongated plasma masses, which, after encystment outside the zoosporangium, give rise to zoospores similar to those of *Saprolegnia*.

*Thraustotheca* <sup>(149)</sup> shows a further stage. The wall of the zoosporangium is fairly thin and the spores at maturity swell up so much that they burst the wall laterally. Some of the spores emerge and gradually all the content forms itself into a rectangular mass attached to the zoosporangium by a thread of protoplasm. The zoospores are kidney-shaped, with laterally placed flagella similar to those secondarily developed in *Saprolegnia*. Thus in *Thraustotheca* we have a form in which the second part of a diplanetic condition is represented. Moreover, this sequence only happens in the presence of pure water. If germinated in a nutrient solution, the spores put out germ tubes without the interpolation of an active phase. If after germination the nutrient solution is removed, the process again becomes modified and the germ tube forms a small sporangium from which a small number of active zoospores are liberated. In fact, these stages may actually take place in the zoosporangium, the spores putting out germ tubes and eliminating any motile stage. We may say therefore that the whole matter of zoospore germination is one of nutrition, that is, a question of physiology. The cutting out of the motile stage occurs in nature in dry weather, while under normal conditions the zoospores swim for about forty minutes and then become encysted. Germination may be delayed for some weeks, but when it does occur, a hypha is formed, giving rise to a sporangium.

The condition in *Dictyuchus* <sup>(150)</sup> is more complex. Here the zoosporangium is so crowded that it forms a mass of hexagonal spores. Each spore is liberated independently from its own area by breaking through the wall. The content flows away and the zoospores when they emerge possess laterally placed flagella. In many respects this condition is comparable with that in *Thraustotheca*.

In the genus *Pythium* we can trace a series starting with a condition like that in *Thraustotheca* and ending with a typical conidium. In *Pythium de Baryanum* the zoosporangium develops a beak-like process at the end of which an additional structure is developed. At maturity the contents of the zoosporangium pass into this beak from which the zoospores escape with laterally placed flagella. The pushing out of this structure may be equivalent to the first motile stage in *Thraustotheca*. Under dry conditions the zoospor-

angium develops like a conidium and itself sends out a germ tube. In *Pythium intermedium* the sporangia are produced in chains and may be discharged before they become motile, or they may function as true zoosporangia and produce separate zoospores.

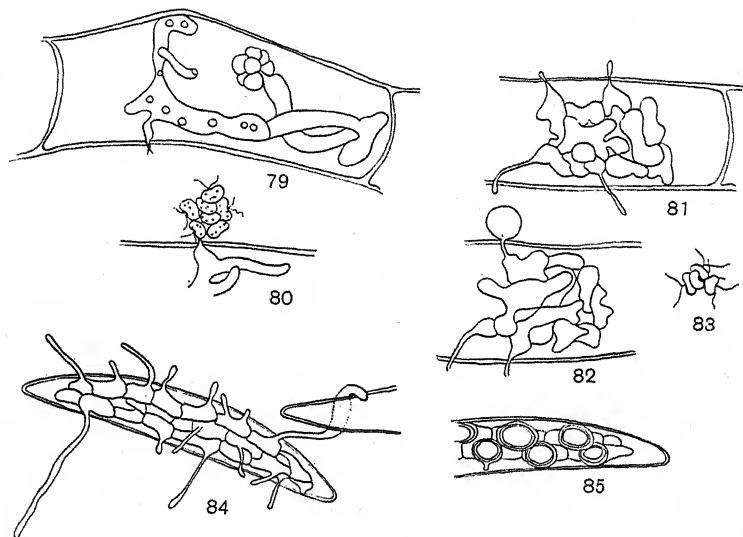
The same condition may occur in the genera *Phytophthora* and *Cystopus* (*Albugo*), where the sporangia are formed in rows, thus giving an increase in spore production.

Von Tavel<sup>(132)</sup> worked out a corresponding case in the Peronosporaceae. In *Peronospora nivea* the contents of the zoosporangium divide up to give a number of biflagellated zoospores. These escape through a terminal papilla. In *Peronospora densa* the protoplasm of the zoosporangium escapes through an apical opening without dividing into separate zoospores. It secretes a fresh wall and germinates directly into a mycelium. In the extreme case of *Peronospora lactucae* the sporangium becomes a spore and on being shed, germinates directly, producing a fresh mycelium. In this species the germ tube is extruded through the remains of the apical papilla, but in *Peronospora radii* no such apical papilla is formed and the sporangium becomes a typical conidiospore.

It can be seen therefore that there is a distinct affinity between the forms in which (a) apical flagella, (b) lateral flagella, and (c) no flagella are developed, and that one type has evolved from another. Atkinson has shown<sup>(6)</sup> in *Pythium intermedium* that the zoospores when first freed from the zoosporangium have laterally placed flagella, but during the swarming period they divide into oval uniflagellated structures with the flagellum at the anterior end. It is clear therefore that it is possible to derive a uniflagellated zoospore from a biflagellated one. It seems reasonable therefore to maintain that flagellation is not a fundamental character of classification as has been held by Butler<sup>(18)</sup> and others. Marshall Ward, Humphrey<sup>(55)</sup> and Butler<sup>(18)</sup> suggest that the kidney-shaped zoospore was most primitive and that the oval spore was an extra stage which has become interpolated. Atkinson<sup>(9)</sup> agrees with this view but in a footnote points out that there is another explanation. In view of the facts as they appear at the present time this latter view seems by far the more reasonable. According to this it is held that the primitive zoospore possesses two apical flagella and in some groups only one. In this condition it germinates and produces a fresh individual. Later a second motile stage becomes necessary and as a result fresh flagella are evolved, and this time laterally. We do not know the advantage derived from lateral flagella, but if any exists, we may

hazard that they have been produced in that position in response to some special requirement.

If the biflagellated zoospore can give rise to a uniflagellated one, it is very probable that the reverse change may also have occurred. Moreover, the biflagellated type of zoospore may have been developed as a direct response to increase in size. It has been generally held that large size or weight of zoospores has resulted in the development of a ring of flagella. This is found in the zoospores of *Oedogonium*.



Text-figs. 79-85. Ancylistaceae

79. *Lagenidium Rabenhorstii*; thallus and prosorangium. (After Atkinson.)
80. *Lagenidium Rabenhorstii*; zoospores forming an exit tube. (After Atkinson.)
81. *Lagenidium americanum*; sporangia with exit tubes. (After Atkinson.)
82. *Lagenidium americanum*; showing the protoplasm passing from one of the sporangia. (After Atkinson.)
83. *Lagenidium americanum*; a group of zoospores. (After Atkinson.)
84. *Ancylistes closterii*; sporangia with exit tubes and one becoming attached to a fresh host. (After Pfitzer.)
85. *Ancylistes closterii*; oospores within the host cell. (After Pfitzer.)

Returning now to the consideration of the Ancylistaceae. In *Lagenidium Rabenhorstii* the mycelium is not extensive but probably contains many nuclei. Reproduction is mainly by means of zoospores which are biflagellated. They are liberated to the exterior through long and frequently coiled exit tubes. Atkinson was able to observe no indications of sexual reproduction in either *Lagenidium Rabenhorstii* (8) or in *Lagenidium americanum* (8), although he had plenty of



material at his disposal. The type of sexual reproduction which has been described in the Ancylistaceae is thought to be more advanced than that in the Chytridiales, and the former may be looked upon as intermediate between the Chytridiales on the one hand and the Pythiaceae on the other. In the higher Phycomycetes only part of the antheridial content passes into the oogonium, whereas in the Ancylistaceae the whole protoplast is transferred into the female organ. The Ancylistaceae are looked upon as intermediate between these two extremes and it is reasonable to consider that their mode of sexual reproduction represents a generalised type rather than the result of a degeneration from a more specialised form.

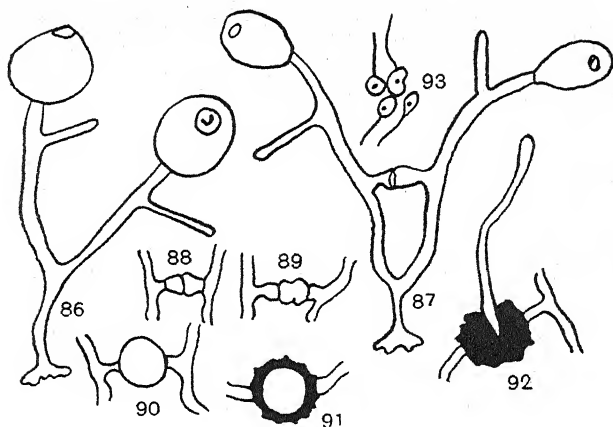
Some five genera are usually placed in the Ancylistaceae, *Lagenidium*, *Ancylistes* (34), *Rhizomyxa* (16), *Protascus* (93) and *Myzocyttium* (156). It has been pointed out elsewhere (28) that *Rhizomyxa hypogaea* shows in many respects a similarity to *Ligniera* among the Plasmodiophorales. Nothing is known of the details of its cytology, and, but for the description of a form of sexual reproduction, this organism might be placed in the Plasmodiophorales. It has been suggested that the sexual structures described in *Rhizomyxa hypogaea* really belong to another organism.

In the migration of zoospores in *Myzocyttium proliferum*, Atkinson sees indications of a resemblance to what is found in *Aphanomyces*, and this genus probably represents the highest development of the Ancylistaceae.

Zopf (157) has suggested that *Rhizidiomyces apophysatus* may be considered as a form relating the Rhizidiaceae with the Ancylistaceae and the Pythiaceae. De Bary considered that the Ancylistaceae were closely related to the Pythiaceae; this view has, in recent years, been well supported and there seems little doubt that the two groups are closely related.

Atkinson (9) has drawn attention to the possibility of deriving both isogamous and heterogamous forms from the Ancylistaceae. He points out that in *Lagenidium Rabenhorstii* there is often little or no difference in the size of the gametangia, the oogonium swelling up at the time of conjugation to form a vesicle which receives the combined gametes. He points out the close similarity between *Lagenidium* on the one hand and *Completozia complens*, a member of the Entomophthoraceae, on the other. He suggests that not only is it possible to derive the higher Oomycetes through the Ancylistaceae, but also the Zygomycetes. With regard to the latter group there is, however, another possible origin.





Text-figs. 86-93. *Zygochytrium aurantiacum*

- 86. Mature plant with sporangia.
- 87. Mature plant showing conjugation of hyphae.
- 88-91. Zygospore formation.
- 92. Germination of zygote to form a fresh hypha.
- 93. Zoospores with a single flagellum.

(All after Sorokin.)

*Zygochytrium aurantiacum* was described many years ago by Sorokin (128) but is only incompletely known. It is found in the tissues of insects, where it forms a yellow mycelium. Sporangia are produced at the ends of hyphae in which swarm spores are developed. They have a single flagellum. Sexual reproduction also occurs: two hyphae come together and fuse, resulting in the formation of a thick-walled zygospore. Unfortunately the further fate of the zygospore is not known, nor is any information available as to the method of infection. In its mode of sexual reproduction *Zygochytrium* shows a remarkable similarity to what is found in the Mucorales, while in its asexual reproduction it may be related to the Chytridiales or Ancylistaceae.

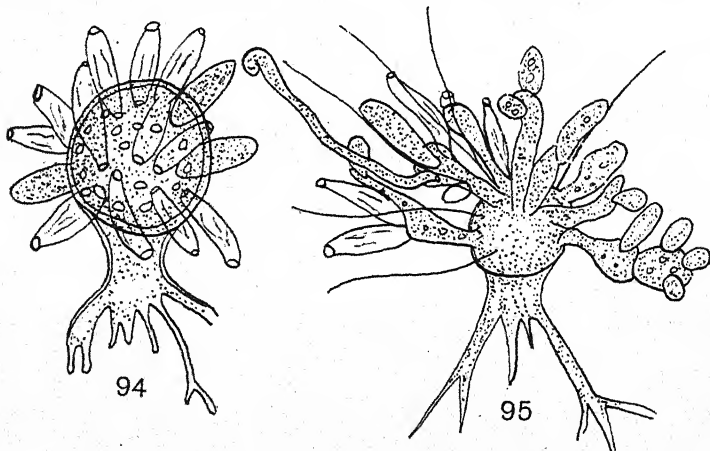
It is to be regretted that more definite information is not available concerning the forms which help to bridge the gap between the Chytridiales on the one hand and the Oomycetes and Zygomycetes on the other. At the same time there is sufficient evidence to justify the conclusion that they were evolved from simpler Chytridiales, although it is not possible to state definitely which these were, nor the exact stages in this slow evolution. As has been pointed out by other workers, it is unlikely that the evolution of the Zygomycetes and Oomycetes from the Chytridiales represents a single phylo-

genetic series. At the same time the accumulation of data relative to the Pythiaceae, the Saprolegniales and the Peronosporales suggests that they were derived from a common ancestor.

#### 8. SAPROLEGNIALES

It is not intended here to consider these higher groups in any detail. Kanouse (71, 72) has shown in her study of these water moulds how they may possibly have been evolved. She suggests that the Peronosporales and Saprolegniales have been evolved from the simpler Leptomitaceae and Blastocladiaceae.

Atkinson has shown how *Monoblepharis* may be related to the Ancylistaceae. *Monoblepharis* has always been a difficulty owing to its motile sperms, and has been used by some workers to relate the higher Phycomycetes with the Algae through forms like *Oedogonium*. Thaxter (136) considered that *Monoblepharis* was more closely allied to *Vaucheria*. Atkinson considers that the antheridia concerned were evolved from zoosporangia, since they resemble them very closely, and that the motile sperms are really comparable with zoospores. As he points out, sex organs in the Archimycetes are generally believed to have originated from sporangia and it is unlikely that those of *Monoblepharis* are an exception to this rule. If one accepts this view then it is not very difficult to relate *Monoblepharis* with the Ancylistaceae on the one hand and the Blastocladiaceae on the other.



Text-figs. 94, 95. *Blastocladia globosa*

94. A typical plant bearing zoosporangia. (After Kanouse.)

95. A plant bearing antheridia, oogonia and zoosporangia. (After Kanouse.)

It is possible to trace a more or less definite series of types from the Blastocladiaceae to the Leptomitaceae. This series consists chiefly in a gradual specialisation of the sex organs and the accompanying formation of more complex zoospores. From the Leptomitaceae there is a definite sequence to the Saprolegniaceae through *Saprolegnia* and *Achlya* to the more complex types like *Dictyuchus*. Regarding the Monoblepharidineae considerable doubt exists as to their correct position. Kanouse (70, 72) considers that the formation of motile antherozoids is primitive and she thinks that the Blastocladiaceae originated from *Monoblepharis*. On the other hand it is quite reasonable to derive the two groups direct from the Ancylistaceae and to consider that *Monoblepharis* is a specialised offshoot which has produced true motile antherozoids by specialisation. If this view is adopted then we trace an evolutionary series from the Chytridiales to the Ancylistaceae and thence to the Saprolegniales through the Blastocladiaceae.

Kanouse considers that the Pythiaceae have been derived from the Leptomitaceae as a specialised parasitic series, but even the Blastocladiaceae appear too specialised to have given rise to the Pythiaceae, with their comparatively simple mycelium, unless one regards them as having degenerated as a result of parasitism. Butler (18) considers that the Pythiaceae originated from the Chytridiales series near the Ancylistaceae. This view seems on the whole preferable, and we look upon the Ancylistaceae as having given rise to the Pythiaceae on the one hand and the Saprolegniales series on the other. Within the Saprolegniales we trace, as Kanouse has done, a series from the Blastocladiaceae to the higher Saprolegniaceae through the Leptomitaceae. From the Pythiaceae we see a series through the Albuginaceae to the Peronosporaceae. It has been

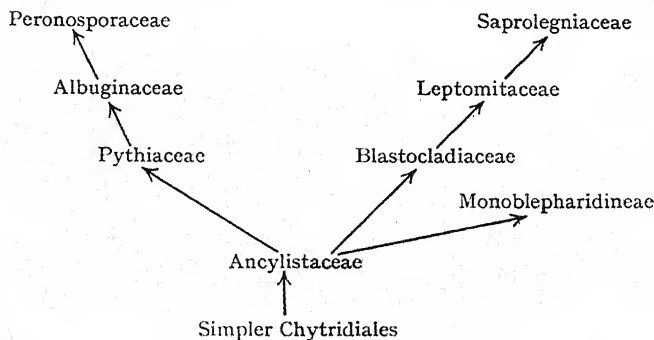


Diagram VI

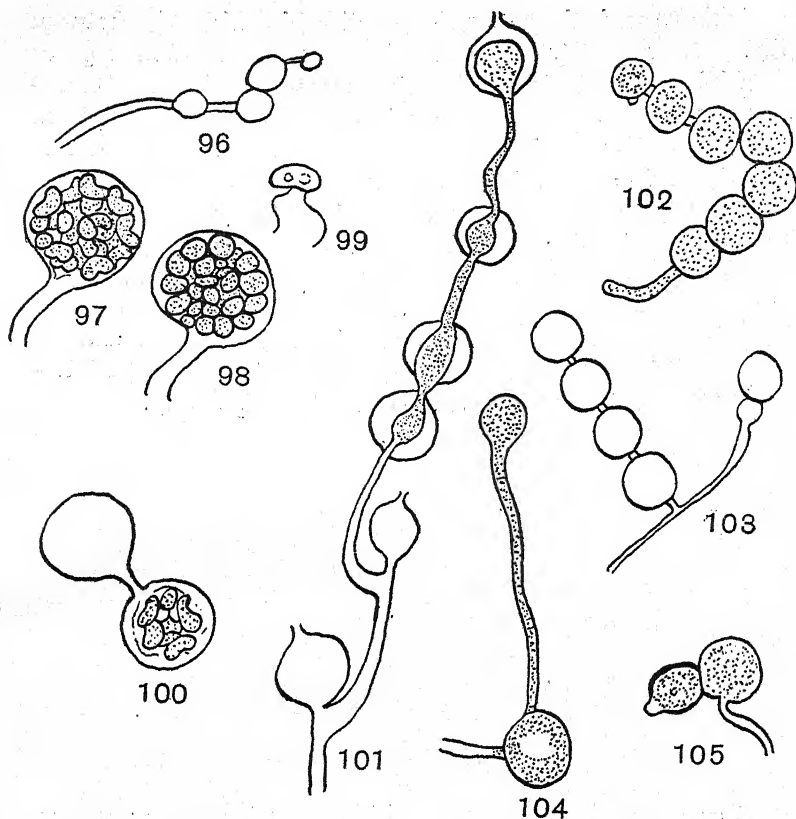
considered that *Dictyuchus* is a specialised form derived from a saprophytic ancestor; this view has been further established by the discovery by Couch (31) of the existence of heterothallism in the genus.

The conclusions discussed above are graphically set out in Diagram VI.

#### 9. PERONOSPORALES

In the Pythiaceae and Peronosporaceae there has been a general and progressive tendency towards the perfection of an aerial method of spore distribution which has resulted in the formation of conidia. This process has been repeated several times, so that we can trace the same sequence of events both in the Pythiaceae and also in the Peronosporaceae. The same is not true of the Albuginaceae which seem to stand apart from the general line of evolution. Von Tavel (133) and others have traced this sequence in the Peronosporaceae and Butler has shown it to occur in the Pythiaceae. It is not necessary here to repeat the evidence brought forward in support of the views expressed by these workers. It is sufficient for our purpose to point out in a general way what is supposed to have taken place. The primitive members of both families possessed motile zoospores. These required water for movement and also a damp situation for germination. The first tendency was to derive a spore which was enclosed within the wall of the zoosporangium when shed and which later was able to germinate by means of zoospores when conditions were favourable. These had their flagella laterally placed and were considered to represent the second part of diplanetism. We have examples of this type in *Pythium de Baryanum* and *Peronospora nivea*. The next stage was the reduction of the sporangium to assist it in distribution, and the evolution of a germ tube mechanism for germination. Thus forms like *Pythium intermedium* and *Peronospora densa* became evolved. Finally we get the reduction of the sporangium to produce a single spore which under normal conditions developed direct by means of a germ tube. This condition is represented by *Pythium ultimum* and *Peronospora radii*. In some of the other genera of the Peronosporaceae the condition has gone further and specialised conidiophores have been developed—a condition which is generally accompanied by reduction in the importance of the sex organs.

The Albuginaceae came from the Pythiaceae presumably after the tendency towards conidia formation had been well established,



Text-figs. 96-105. Pythiaceae

- 96. *Pythium gracile*; hypha bearing sporangia in a conidial-like condition.
- 97, 98. *Pythium gracile*; zoospore formation.
- 99. *Pythium gracile*; mature zoospore.
- 100. *Pythium proliferum*; zoospore liberation.
- 101. *Pythium proliferum*; showing the proliferation of the zoosporangia.
- 102, 103. *Pythium intermedium*; a chain of sporangia or conidia.
- 104. *Pythium intermedium*; germination of a conidium without falling off its stalk: it is producing a new spore at the tip of the germ tube.
- 105. *Pythium vexans*; conidium budding off another laterally.

(All after Butler.)

at any rate they have left no forms which were dependent upon zoospores for asexual reproduction. This method of sexual reproduction more closely resembles that of the Pythiaceae than the Peronosporaceae and it is therefore generally held that the Albuginaceae have been derived from the Pythiaceae rather than from the Peronosporaceae.

Ashby (3) has indicated the close relationship which exists between *Phytophthora* and *Pythium* and points out that the former genus should be grouped with the Pythiaceae and not with the Peronosporaceae as is done by some writers. The genus *Trachysphaera* has probably been derived from *Phytophthora*, and should therefore be included in the Pythiaceae and not in the Peronosporaceae.

The parallel evolution of the conidium may be represented graphically as in Diagram VII. Such a tree shows the way in which the two families have developed by parallel evolution, and indicates the common ancestry of the two families. It is considered that the Albuginaceae were derived from this series from a common and not very specialised member, but owing to the entire absence of intervening types it is impossible to see its relationship clearly.

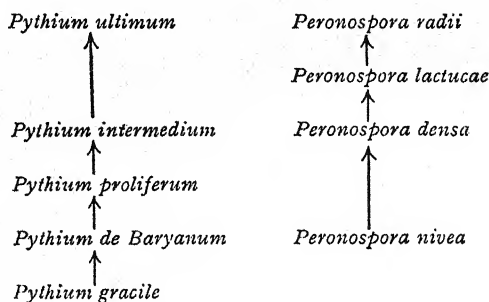
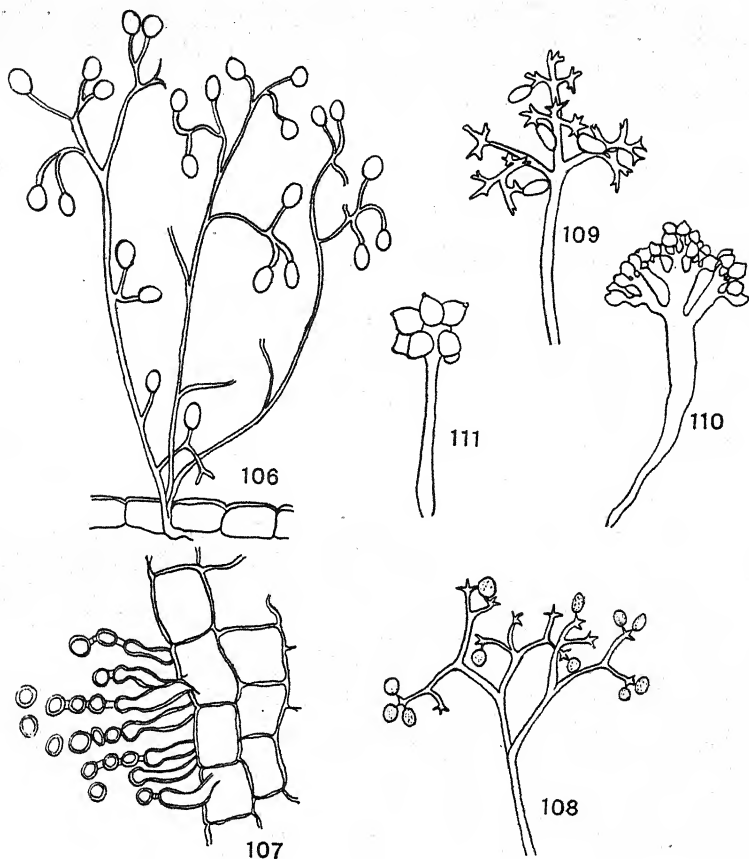


Diagram VII

The relationships of the genera within either the Peronosporaceae or the Saprolegniaceae scarcely concern us here. The forms with which we are familiar are in most instances specialised parasites on Phanerogams. They have lost as a result of their specialisation the characters which would enable us to relate them to one another, and any view expressed as to their interrelationship must be very tentative. There is some indication from the character of the conidiophores that there is a series from *Peronospora* through *Bremia* to *Sclerospora* and then to *Basidiophora*. *Pseudoperonospora* does seem to stand apart and to be more closely related to *Peronospora*. In the Saprolegniaceae it is even more difficult to find criteria to give evidence of any evolutionary series, and probably little advantage is gained by attempting to suggest one.

It is desirable at this point to make some reference to the Bacteria. The current work on that group seems to remove them farther and farther from the rest of the fungi. They appear to have little in



Text-figs. 106-111. Peronosporales

106. *Plasmopara nivea*; conidiophores.  
 107. *Cystopus candidus*; chains of conidia.  
 108. *Bremia lactucae*; conidiophore.  
 109. *Peronospora leptosperma*; conidiophore. (After de Bary.)  
 110. *Sclerospora graminicola*; sporangiophore. (After Weston.)  
 111. *Basidiophora entospora*; sporangiophore. (After Roze and Cornu.)

common with them except the absence of chlorophyll. The recent work on various diseases and ultramicroscopic bacteria, too, suggests that their origin may be quite independent of the rest of the fungi. Whatever may be the ultimate decision on this point we are fairly safe in concluding that they have played no part in the evolution of the Archimycetes, that they are not even distantly related to them, and therefore do not concern us here.

Mention must also be made of the Myxobacteriaceae. Their relationships are very obscure, particularly as little or no cytological work has been done on them. It is held by some that they are related to the Bacteria, but this may quite probably be a purely superficial resemblance and not one of phylogeny. They have also been related to the Cyanophyceae. Recent work on the latter group has, however, not cleared up the position of the Cyanophyceae to any appreciable extent. Whatever be their origin it seems certain that they are in no way closely related to the Archimycetes.

#### 10. SUMMARY

Summarising the views expressed regarding the series of the Chytridiales we may say that starting from a primitive *Proteomyxa* ancestor it is possible to trace a series which shows first the development of a sporangium, and a method of distribution of its asexual products; as development became more complicated a more advanced soma was evolved, firstly in order to provide the fungus with food and later to enable it to be self-supporting and to provide it with a method of attachment. Concurrently with this we find the evolution of sex organs, first the fusion of motile isogametes, closely resembling the zoospores, later the development of true sex organs which at no time possessed motility, although their product may at times have been provided with means of locomotion. We have seen that there is strong evidence that the development of true antheridia and oogonia has occurred more than once even within the Chytridiales, and that those developed have no phylogenetic relationship with those found within the Algae.

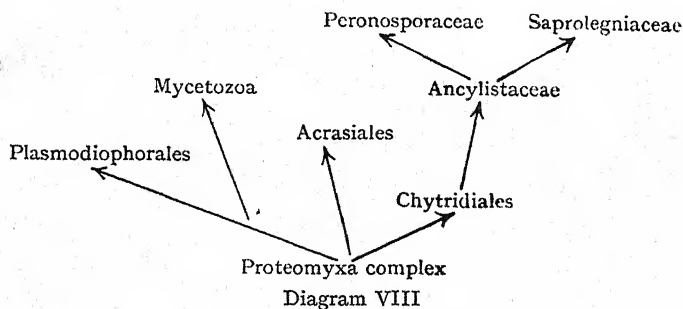
As we pass from the Chytridiales to the Ancylistaceae we find sexual reproduction becoming more complicated, and in the Saprolegniales we have the final expression of an antheridium and an oogonium. In the Peronosporales we find parasitic and saprophytic forms; these have become adapted to terrestrial life. Motile zoospores have been replaced by wind-borne conidiospores, and oospore formation has to a large extent lost its importance.

As has been previously pointed out, the series is by no means clear. We possess at the present time only the remains of what may have once been a more widely distributed and important group. We know practically nothing of fossil forms. In the Rhynie fossils fungi have been found, though their systematic position is very doubtful. Some of these have been described by Kidston and Lang (76). They appear to be related to the Chytridiales or the Peronosporales.



Many are remarkable for the size of the sporangia, which are larger than any species known at the present time. This size, however, may be related to the conditions under which they were living. We have attempted to show, however, that using the types which are known to us at the present time we can obtain sufficient evidence to indicate how the whole group may have been evolved, and what are the probable stages in this evolution. Many more species of Chytridiaceous fungi still await description, and many of those already described require much more critical study. When this has been done we may be more able to fill in the gaps in their evolutionary series.

It is considered that the Peronosporales, Mycetozoa, Acrasiales and Chytridiales have been evolved separately from the *Proteomyxa* complex in a way similar to what has been suggested by Fritsch (44) as the origin of the algae. These conclusions are graphically set out in Diagram VIII.



The observations set out in this paper are not intended to be by any means final; they are put forward rather as suggestions. Further work on the group alone will show how much of these views is in accordance with fact. It is hoped that more workers will take up the study of what is still a very little explored group.

My thanks are due to Professor W. T. Gordon for permission to reproduce photographs from slides of the fungi in Pl. VIII, figs. 9-12, and to Mr S. F. Ashby for the loan of the slide from which Pl. VIII, fig. 5 was taken.

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## ERRATUM

In the first instalment of this paper (*New Phyt.* xxvii, 3, p. 252) text-figs. 33 to 36 should be described as of *Rhizophidium globosum*, and 37 to 40 as of *R. brevipes* instead of vice versa.